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1

ASSOCIATIONS BETWEEN PROTECTION FROM MALARIA AND ANTIBODIES TO KNOWN AND PREDICTED MEROZOITE ANTIGENS

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Antibodies play an important role in protective immunity against *Plasmodium falciparum* in humans. Merozoite antigens are likely to be important, but the major targets mediating protection have not been clearly identified. Very few of the large number of merozoite antigens have been studied as targets of human immunity, and few prospective cohort studies have compared responses to a multitude of antigens. In this study we aimed to assess the acquisition of antibodies and protective associations for most merozoite antigens that are regarded as potentially important targets. We screened 139 recombinant proteins that were either known or predicted to be *P. falciparum* merozoite antigens located on the merozoite surface or in apical organelles. After assessment of antigen quality and immunoreactivity, 75 proteins were tested for antibody responses using plasma from a prospective cohort of 206 school-aged children resident in Papua New Guinea. For each antigen, we assessed the acquisition of antibodies to merozoite antigens by examining associations with age, exposure, and active infection, and we prospectively examined associations between antibodies and protective immunity. Antibody responses to almost all merozoite antigens were associated with reduced risk of malaria. However, the strength of protective associations varied substantially between antigen-specific responses, which may reflect their significance as targets of protective immunity. Protection from malaria is likely to result from a combination of responses to different antigens. Examining this, we found that responses to specific combinations of antigens were most strongly associated with protection, which supports the strategy of including multiple antigens in a vaccine. These findings have important implications for understanding and evaluating human immunity, and for the selection of specific candidate antigens for vaccine development.

2

EARLY PRODUCTION OF HIGH AVIDITY ANTIBODIES TO FULL-LENGTH VAR2CSA DURING PREGNANCY CORRELATES WITH ABSENCE OF PLACENTAL MALARIA

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Pregnant women, especially primigravidae, are at risk of *Plasmodium falciparum* malaria because infected-erythrocytes (IE) sequester in the placenta causing placental malaria (PM). Sequestration is mediated by VAR2CSA on IE that binds to chondroitin sulfate A (CSA) on placental cells. In the placenta, IE induce inflammation and monocyte infiltration that increases the risk of maternal anemia and poor birth outcomes. Antibodies (Ab) to VAR2CSA can block sequestration and have been associated with improved pregnancy outcomes. However, a direct link between Ab levels to VAR2CSA and clearance of parasites from the placenta resulting in absence of PM at delivery has not been reported. The role of high avidity Ab in clearance of placental IE is also unknown. The goal of this study was to identify IgG responses to full-length VAR2CSA (FV2) that correlate with absence of PM. Using the bead-based multi-analyte profiling assay, Ab levels to FV2 in 89 women living in high and low transmission areas in Cameroon were measured using samples collected during the course of pregnancy. The percentage of high avidity Ab to FV2 (i.e., percent Ab bound in the presence of 3M NH_4SCN) was determined. In the high transmission area, the level of Ab to FV2 ($p=0.0047$) and the percentage of high avidity FV2 Ab ($p=0.0009$) were significantly higher in women without PM than those with PM. Further, women with moderate FV2 Ab levels in the 5-6th and 7-8th month had a 2.3 (95% CI, 1.0-4.9) and 2.0 times (95% CI, 1.0-3.9), respectively, reduced risk of PM at delivery. Also, women who had ≥35% of high avidity Ab to FV2 at 5-6th month had a 7.6-fold lower risk of PM ($p=0.0013$, 95% CI: 1.2-50.0). In contrast, no difference was found in women living in the low transmission area. Differences between the two study sites show that frequent malaria infections are required to develop protective Ab. In conclusion, early production of Ab to FV2, especially those with high avidity, are associated with absence of PM.

3

MOTHER AND NEONATE DISTINCT IMMUNOGLOBULIN G: A NEW APPROACH USING PROTEOMICS FOR NEONATAL SEROLOGICAL DIAGNOSIS

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This study provides for the first time a way to distinguish neonatal from maternal antibodies and to measure specific antibodies synthesized by a newborn. In the context of malaria, the knowledge of firstly acquired antibody responses against *Plasmodium falciparum* is essential for orientating the choice of appropriate vaccine strategies. Nevertheless, as maternal antibodies are transferred to the fetus during pregnancy, shared maternal and neonatal antibodies are present in the infant's plasma during his first months of life. We propose a technique of differential detection and dosage, in newborn plasma, of immunoglobulin G of mother and child, by a proteomic approach. This method relies on the allelic polymorphism of the IgG3 that corresponds to thirteen G3m allotypes located on the constant domains of the heavy chains. Peptide sequences encompassing G3m discriminatory amino acids, aimed at identifying the greatest number of G3m allotypes, were defined. Preliminary experiments were done on a series of controlled mixtures of plasma samples from individuals homozygous for distinct G3m allotypes, as determined by a classical haemagglutination-inhibition method: total IgG3 were purified using affinity chromatography before being digested by a combination of proteases; resulting peptides were separated by nano-HPLC and allotype-specific peptides were successfully detected by mass spectrometry. A label-free approach using the nano-HPLC retention times and peak intensity of the peptides gave semi-quantitative information showing a significant correlation with the artificial allotypes-mix ratio. Validation of the proteomic approach was made on total IgG3 purified from plasma

samples of one mother and her baby drawn quarterly from birth to nine months. The concomitant serological determination of the father's Gm allotypes allowed determining unambiguously the G3m allotypes of the infant. The possibility of quantifying neo-synthesized total IgG3 in infant, offered by this new method, may be extended to specific IgG3 elaborated in response to pathogens. It will allow improving knowledge on the acquisition of anti-malarial natural immunity in infancy. In a wider perspective, this approach represents a promising diagnostic tool for vertically-transmitted diseases.

4

DECREASED HUMAN ANTIBODY RESPONSE AGAINST *PLASMODIUM FALCIPARUM* ANTIGENS EXPRESSED IN BOTH GAMETOCYTES AND GAMETES

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The role of natural immunity in malaria transmission is complex, but critical to disease control efforts. Development of the sexual stages of the *Plasmodium* parasite that are required for transmission begins in RBCs in the human host. After maturation gametocytes circulate for several days before being cleared by the human host if not taken up in a blood meal by a mosquito. In the mosquito midgut the gametocytes emerge from the RBC as extracelluar gametes which fertilize and begin sporogonic development. The surface of the extracellular gamete is a target for malaria transmission-blocking antibodies and four antigens (Pfs230, Pfs48/45, Pfs25 and Pfs28) have been identified and are being developed as vaccine candidates. Pfs25 and Pfs28 are only translated in the mosquito, but Pfs230 and Pf48/45 are expressed in the gametocyte and therefore exposed to the human immune response. To examine antibody production against antigens expressed on sexual stages, proteomic data from gametocytes and gametes was incorporated into the analysis of the data from a recombinant *P. falciparum* protein microarray probed with plasma from 220 individuals before and after the malaria season in Mali. The results indicate that antibodies against antigens represented on the array that are expressed in gametocytes or both gametocytes and gametes, including Pfs230 and Pfs48/45, increased with age and from the beginning to end of the malaria season. This finding is consistent with exposure to sexual stage parasites during the course of the season, which could boost a transmission-blocking vaccine. Interestingly, analysis of immunogenic antigens indicated that there was a significantly stronger antibody response against antigens expressed in gametocytes, than those expressed in both gametocytes and gametes. This decreased response against antigens expressed in gametes was evident at the both the start and end of the season ($p<0.008$ and $p<0.0002$, respectively) and suggests a bias against antigens that could interfere with malaria transmission.

5

ANTIGEN-SPECIFIC MEMORY B CELL DETECTION USING A FLOW CYTOMETRY BASED ASSAY

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Infant B cell development and memory formation is poorly understood because B cell frequency in peripheral blood is low and there is limited sample volume of blood that can be obtained from infants. To address this limitation, we have developed a flow cytometry based assay capable of detecting antigen specific memory B cells from small volumes of peripheral blood. We first developed and validated this assay using tetanus (TT) and diphtheria (DT) vaccine responses. The flow-based assay is 1.5 - 4 times more sensitive at detecting TT and DT-specific memory B cells at higher frequencies compared to the traditional B cell ELISPOT assay. Moreover, because this assay can be multiplexed, a total of 10^6 PBMC are consumed for TT and DT specific memory B cell detection with the flow based assay compared with $2x10^6$ PBMC needed for the DT B cell ELISPOT alone (10^6 PBMC are needed for each additional antigen tested by ELISPOT). We have applied this technology to cord blood mononuclear cells and successfully detected TT and DT-specific memory B cells in neonates whose Kenyan mothers were vaccinated during pregnancy. We have recently expanded this assay to detect malaria antigen-specific memory B cells. Specifically we were able to detect MSP1, MSP3, but not AMA1 specific memory B cells from 5 Kenyan adults and 1 Kenyan child (age 28 months) with known past malaria infection. 1 Kenyan child (age 28 months, same region) with no evidence of past malaria infections (no T cell responses to MSP1 and no antibody recognition of multiple malaria antigens by serology) had no detectable malaria antigen-specific memory B cells using the flow assay. Additionally, we were able to detect MSP1, MSP3, but not AMA1 specific memory B cells from a neonate whose Kenyan mother had evidence of malaria during pregnancy, indicative of fetal priming to malaria antigens. These data suggest that this flow based assay will be a valuable tool to overcoming major constraints and furthering our understanding of the development of human infant B cell immunity.

6

ASSOCIATION OF HLA ALLELES WITH *PLASMODIUM FALCIPARUM* SEVERITY IN MALIAN CHILDREN

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Pre-erythrocytic immunity to *Plasmodium falciparum* malaria is likely to be mediated by T cell recognition of malaria epitopes presented on infected host cells via class I and II major histocompatibility complex (MHC) antigens. To test for associations of HLA alleles with disease severity, we performed high resolution typing of HLA class I and II loci and compared the distributions of alleles of HLA-A, -B, -C and DRB1 loci in 359 Malian children of Dogon ethnicity with uncomplicated or severe malaria. We observed that alleles A*30:01 and A*33:01 had higher frequency in the group of patients with cerebral disease compared to patients with uncomplicated disease (A*30:01: gf=0.2031 vs. gf=0.1064, OR=3.17, P=0.004, CI [1.94-5.19]) and (A*33:01: gf=0.0781 vs. gf=0.0266, 4.21, P = 0.005, CI [1.89-9.84]), respectively. The A*30:01 and A*33:01 alleles share some sequence motifs and A*30:01 appears to have a unique peptide binding repertoire compared to other A*30 group alleles. Computer algorithms predicted malaria peptides derived from Liver Stage Antigens 1 and 3 (LSA-1 and LSA-3), Merozoite Surface Protein 1 (MSP-1) and Thrombospondin-related Anonymous Protein (TRAP) with strong

binding affinity for HLA-A*30:01 and HLA-A*33:01 but not to a closely related allele A*30:02. In conclusion, we identified A*30:01 and A*33:01 as potential susceptibility factors for cerebral malaria providing further evidence that polymorphism of MHC genes results in altered malaria susceptibility.

7

HIV-MALARIA CO-INFECTION: CHARACTERIZATION OF THE IMMUNE MECHANISMS AT PLAY

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The majority of malaria cases, like HIV, occur in sub-Saharan Africa, where many individuals are infected with both pathogens. Co-infection with *Plasmodium falciparum* malaria and HIV is a growing concern, as co-infected individuals experience higher parasite burdens and worse clinical outcomes. However, the underlying mechanisms responsible have yet to be fully investigated. Robust pro-inflammatory innate host immune responses are key to resolving malaria infection. IFNγ produced by NK, NKT and γδ T cells is required to control parasitemia. We hypothesized that HIV co-infection compromises the function of these innate immune cells in response to malaria. Our aim was to examine the inflammatory response of fresh peripheral blood mononuclear cells (PBMCs) isolated from therapy naïve HIV-infected donors to malaria parasites, and to determine whether highly active antiretroviral treatment (HAART) impacts these responses. Compared to HIV- individuals, PBMCs from patients with chronic HIV infection showed a marked decrease in the percentage of Th1 (IL18R+) NK, NKT and γδ cells, with only the percentage of IL18R+ NKT cells improving after six months of HAART in the HIV+ patients. In response to malaria parasites, production of IFN-γ by PBMCs, particularly NK, NKT and γδ T cells, was greatly decreased in HIV patients after 2 days of incubation; levels did not improve when retested after six months of HAART. This was also true for two other cytokines implicated in the response to *P. falciparum*: IL-2 (measured on day 2), as well as TNF (measured on day 1). Interestingly, HIV patients could mount strong inflammatory responses to PMA. In conclusion, we suggest that HIV infection impairs the inflammatory response of innate effector cells to *P. falciparum* malaria; we are currently investigating the roles of IL-10, TGF-β and TIM-3 in this process. We believe that the altered innate immune response contributes to higher parasite burdens and ineffective immune responses in co-infected individuals.

8

INFLUENCE OF THE TIMING OF MALARIA INFECTION DURING PREGNANCY ON BIRTH WEIGHT AND ON MATERNAL ANEMIA IN BENIN

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Malaria in pregnancy (MiP) is a threat both to these mothers and to their babies. Indeed, MiP increases the risk of maternal anaemia and of low birth weight (LBW). LBW is the single most important determinant of mortality during the first year of life in African infants. It has been estimated that between 62,000 to 363,000 newborn deaths occurred each year as a direct result of LBW due to malaria in pregnancy. Although consequences of malaria in pregnancy are well known, the period of pregnancy in which infection has the highest impact is still unclear.The characterization of the most harmful period of malaria infection during pregnancy will help to improve preventive policies. In Benin, we followed-up a cohort of 1037 women through pregnancy until delivery. Women were encouraged to consult early and ultrasound scans were performed to assess accurately the gestation age. The objective was to evaluate the relationship between the timing of infection and birth weight, and maternal anaemia at delivery. At the beginning of pregnancy (before 4 months of gestation), peripheral infections were associated with a decrease in mean birth weight (-98.5gr; p=0.03) and an increase in the risk of anaemia at delivery (aOR=1.6; p=0.03).Infections in late pregnancy (during the third trimester of pregnancy) were related with a higher risk of maternal anaemia at delivery (aOR=1.7; p=0.001). Placental infection and the number of malaria infections occurring during this period were also associated with a higher risk of maternal anaemia. These findings may have important implications for the management of MiP, as women in such settings often consult late during their pregnancy and therefore remain unprotected in early pregnancy. Malaria infections at the beginning of pregnancy seem to have major effects, both in terms of birth weight and maternal anaemia. As the sulfadoxine/pyrimethamine intermittent treatment for prevention of MiP is given during the second trimester and women are seen late in pregnancy, they stay unprotected during the early period of gestation. To fully protect the women through the whole duration of pregnancy, additional measures should be put forward, such as the use of impregnated bed nets and appropriate treatment of malaria infections. In the future, a vaccine against pregnancy-associated malaria parasites could protect the women in the early pregnancy, which seems to be a high risk period.

9

HETEROGENEITY OF MOSQUITO EXPOSURE IN AFRICAN VILLAGES: STABILITY OVER TIME AND IMPORTANCE FOR MALARIA CONTROL

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Exposure to *Anopheles* mosquitoes is highly variable within African villages; mosquito densities vary over time and between households. This heterogeneity in contact between humans and mosquitoes is an important factor in determining the spread of malaria and can influence the efficacy of transmission reducing interventions considerably. However, micro-epidemiological differences in mosquito exposure are poorly quantified and even less is known about the stability of mosquito hotspots over time. We longitudinally determined mosquito exposure in 2 villages in Mali and 8 villages in Tanzania, representing transmission settings ranging from very low and seasonal to intense perennial malaria transmission. In each of the villages 30-298 households were sampled with standard light traps during 3-52 nights. We observed that mosquito exposure was highly consistent in some villages exposed to perennial transmission. Although mosquito densities were reduced by more than 10-fold in the dry season, households that were exposed to the highest mosquito densities in the wet season were the same as those in the dry season. In contrast, in a village exposed to intense seasonal malaria, we observed no consistency in mosquito densities over time or any geographical patterns. We incorporated our findings of heterogeneity in mosquito exposure in a previously validated microsimulation model to simulate its influence on the impact of malaria transmission blocking vaccines (MTBV). We assumed a vaccine efficacy of 90% in preventing mosquito infection from human gametocyte carriers; 70% coverage in the total population and coverage with insecticide treated nets and artemisinin combination therapy as indicated by our village surveys. A strategy that would include all hotspots of mosquito exposure, the 20% of households exposed to the highest mosquito densities, would have a 2-fold higher impact on human parasite prevalence compared to random coverage. Strikingly, the impact of a

MTBV would be largely dissolved if hotspots of mosquito exposure would be missed. Findings will be discussed in the light of operational tools to identify hotspots of mosquito exposure

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CHARACTERISTICS OF MALARIA HOTSPOTS IN A MODERATE ENDEMIC SETTING IN NORTHERN TANZANIA

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Transmission of malaria is highly heterogeneous and is clustered even in areas of moderate transmission with groups of households, termed hotspots, maintaining high transmission throughout the year. Hotspots provide a reservoir of parasites for mosquitoes that spread the infection outside the immune populations in the wet season. Hotspots are not well defined. In this study we aimed to identify and describe the characteristics of hotspots in a moderate transmission setting in northern Tanzania. A complete household survey was carried out in 4 villages in Misungwi district, Tanzania from September to November 2010. Every household was visited and mapped by GPS. From consenting households morbidity, malaria risk and demographic data were collected. All people present were further consented to give a dried blood spot that was stored and later analyzed for *Plasmodium falciparum* using a nested PCR. Passive surveillance of fever cases at 3 health facilities were tested with an RDT to confirm malaria. These were treated with an ACT and visited at home in order to establish the GPS position. Cross sectional survey and health facility surveillance data were combined and analyzed for clustering. A total of 1610 people from 662 households were included in this analysis. 3 hotspots were identified, median size of 170m (range 95-185) radius and contained 113(7%) people and 24(3.8%) households. Those living in hotspots compared to outside were significantly more likely to be parasitemic (OR 2.1, p>0.001), live more than 30 minutes walk from a health facility (OR 2.2,p>0.001), have significantly more open eaves (OR 1.2 p>0.001), were of lower socio-economic status (OR 1.6, p>0.001) and were less well educated(OR 2.6 p>0.001). Results from passive surveillance showed that the odds of a case of malaria coming from within hotspots were triple compared to outside a hotspot (OR 3.35, 95% CI 1.42-7.89, p=0.006). In conclusion, this study highlights the importance of hotspots for malaria transmission and control, and that a small proportion of the population bear the highest burden of malaria. For the first time the populations within a hotspot are described in detail. Hotspots are small and contain households with known risk factors for malaria. It is likely that these hotspots are responsible for on-going transmission in the dry season and should be the target for future malaria control efforts.

11

HOW LITTLE CAN BE MEASURED? MONITORING *PLASMODIUM FALCIPARUM* TRANSMISSION INTENSITY USING SERO-EPIDEMIOLOGICAL COHORT STUDIES

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At very low intensities of malaria transmission obtaining reliable estimates of transmission intensity becomes progressively more difficult because parasite-positive individuals can rarely be found. Such situations may arise naturally or as consequence of a successful control- or elimination program. Since antibody responses to malaria are long-lived, serological data contains information on past exposure and holds the key to detecting minuscule amounts of transmission. The perhaps most sensitive approach to estimating transmission intensity makes use of age-seroprevalence curves, effectively integrating information over decades of exposure. However, when monitoring malaria control- or elimination efforts, the force of infection which is currently acting may be of more interest than the force of infection in the past. Measurement of current transmission intensity requires collection of either repeated cross-sectional or cohort-data. Using data on ama1 and msp2 antibody titers from 160 Indonesian schoolchildren, collected over 5 months, we demonstrate how to make efficient use of longitudinal serological measurements (cohort data). Rather than considering age-prevalence curves, we use the longitudinal information contained in the changes of titer over time to identify individuals that were never exposed. These we use to assign a probability of being positive to all samples which allows subsequent calculation of the force of infection during the study period. The dataset showed an estimated force of infection of ca. 0.06 person-1 year-1, which is at the lower range of what is identifiable with the utilized study design. Using simple calculations, we show how the size of the study population, the intensity of transmission and the study duration influence the reliability of force of infection estimates obtained in the described manner.

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CHARACTERIZING PATTERNS OF MALARIA TRANSMISSION RISK IN CAMBODIA USING SEROLOGICAL AND PARASITOLOGICAL DATA

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The spatial distribution of malaria in Cambodia is patchy and discontinuous and this heterogeneity represents a major challenge to planning and implementing malaria control activities. All villages in Cambodia are individually assigned a malaria risk category based mainly on distance to forest. This policy reflects operational experience on the part of the control programme. It is also supported by evidence from empirical studies carried out in the Mekong region, although relatively few studies are specific to Cambodia. Since 2004, Cambodia has carried out a series of national malaria surveys. Sampling within these surveys is stratified according to distribution of forest at national level and all households sampled in these surveys are mapped. These data therefore offer a unique opportunity to characterize geographical patterns of malaria transmission in Cambodia and to assess the validity of current approaches to targeting control activities. This paper presents an analysis of individual-level parasitological and serological data from the 2004 Cambodia national malaria survey. Results from multivariate analyses showed a significant positive relationship between individual risk of infection/antibody rates and proximity to forest after controlling for confounding factors. Results also indicated substantial levels of infection beyond the national control programme's high/medium risk zones, prompting a subsequent expansion of existing risk zones and associated interventions. As antibody prevalence to malaria antigens was approximately ten times higher than parasite prevalence, serological outcomes provided a highly sensitive marker of transmission intensity and provided specific insights into transmission dynamics. Individuals over 15 years and who visited the forest, for example, had significantly elevated antibody rates. In addition, a number of villages that were entirely negative for parasites exhibited high antibody prevalence, suggesting high historical levels of transmission or possible foci of infection undetected by parasitological measures.

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MORE THAN JUST A HOLIDAY: SEVERE IMPORTED *PLASMODIUM FALCIPARUM* MALARIA IN THE UK - EPIDEMIOLOGY AND MANAGEMENT

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Malaria remains a significant global challenge with an estimated 225 million cases and 781,000 deaths in 2009. In the UK, a statutory notification system captures 66% of *Plasmodium falciparum* cases; 1495 imported *P. falciparum* infections were notified in 2010. People of African origin visiting friends and relatives (VFRs) represent a significant proportion of cases in other European countries and may be an important and under-recognised target group for prophylaxis. Severe malaria is avoidable either by use of appropriate prophylaxis or prompt access to treatment; intravenous artesunate is now the WHO-approved first line therapy. We conducted a retrospective observational study of severe malaria in the UK during 2008 to describe the epidemiology and audit clinical management. Notification data from the Health Protection Agency Malaria Reference Laboratory (MRL) were used to identify cases of severe and possible severe *P. falciparum* from 2008 (WHO criteria). Full clinical information was then sought and we report on the subset of 112 (10.3%) confirmed severe cases. The largest group were Black Africans (71%), male (58.9%), age 17-65 years (78.4%), with peak frequencies in June and December (26.8%). Caucasians accounted for 18.8% of cases. Most infections were acquired in West Africa (83.9%); 50% of cases travelled to see relatives, 20.2% were on holiday. Only 17.5% of VFRs and 20% of holiday-makers took appropriate prophylaxis. 97.5%% of all cases received in-patient treatment, but only 59.3% were treated according to best practice (56.3% of VFRs, 61.5% of holiday makers). All 5 deaths were in-patients who were treated inappropriately. Our study therefore highlights the insufficient use of prophylaxis in all groups of travellers, and shows that Caucasian holiday-makers are at significantly greater risk of severe disease (19.4% vs VFRs 9.5% p=0.008). It also exposes serious deficiencies in the management of imported severe *falciparum* malaria in the UK.

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SEROLOGICAL TESTING OF DONORS WITH HISTORY OF MALARIA AND TRAVEL TO MEXICO

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The American Red Cross loses ~93,000 donations/year to malaria deferrals for travel to and/or residence in an endemic area or history (hx) of malaria. Most deferrals are for travel, often to low risk areas (e.g., Mexico). Since 1998, only 5 cases of transfusion-transmitted malaria (TTM) were reported in the US, all attributed to past infected residents of Africa. To evaluate current transmission risk, we tested malaria deferred donors correlating antibody results with a hx of malaria or travel to Mexico. Since 2006, malaria deferred donors in a single ARC region were enrolled in the study, including all donors with hx of malaria or past residence, and randomly selected travel deferred donors. Enrolled donors provided 2 tubes of EDTA blood and completed a *Plasmodium* exposure questionnaire. Blood samples were tested for *Plasmodium* antibodies by EIA (Lab21 Healthcare). Repeat reactive (RR) samples were considered positive and tested by Real-Time PCR. Questionnaires were analyzed for country of birth, travel history, past hx of malaria and other risk factors. Of 4,877 donors enrolled, 77 (1.6%) were RR. Irrespective of deferral type, 40 (52%) of 77 RR had a past hx of malaria, including 27 (50%) of 54 RR travel deferred donors. Four (5.2%) RR donors were PCR+, 2 had hx of malaria. Of the 4,687 travel deferrals, 983 (21%) visited Mexico; only 1 (0.1%) was RR, but this donor was infected in Turkey. Of the 983 Mexico deferred donors only 22 (2.2%) traveled to Chiapas/Oaxaca (high risk), while the vast majority (n=948, 96%) traveled to areas with little or no malaria risk (i.e., Cancun). Semi-immune donors have been linked with most TTM cases and pose a threat to the blood supply. Donors with a previous hx of malaria, regardless of deferral category, could be semi-immune as indicated by serological testing in our study. In contrast, only 2.2% of donors traveling to Mexico visited high risk malarial areas. Thus, consideration should be given to permanently deferring donors with past hx of malaria, while limiting deferrals for travel areas of Mexico with little or no risk.

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THE EPIDEMIOLOGY OF CHOLERA IN PAPUA NEW GUINEA

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An estimated 10,000 cases of cholera have occurred in Papua New Guinea (PNG) since mid-2009. Cholera is endemic in many countries in the Asia-Pacific region; however, this is the first documented outbreak in PNG. Little is known about the disease in the PNG context; thus, we investigated the epidemiology of cholera through molecular typing of pathogenic *Vibrio cholerae* isolates. Isolates were obtained from culture confirmed cholera cases. We conducted variable number tandem repeat (VNTR) analysis, multilocus sequence typing (MLST), cholera toxin prophage (CTXϕ) molecular typing and phenotypic tests to characterise *V. cholerae* isolates. Although only a small proportion of suspected cases have been culture-confirmed, we obtained isolates (n=12) from five locations throughout lowland PNG have been comprehensively characterized thus far. MLST analysis suggests that PNG isolates are most closely related to isolates previously reported in Vietnam and Bangladesh. VNTR analysis revealed all isolates to be clonal, with affinities to Vietnamese strains. On the basis of CTXϕ and genomic analysis the PNG clone has an altered structure. The clonal origin and homogeneity of PNG *V. cholerae* strains suggests the outbreak is the result of a recent incursion. Interpreting relatedness of PNG isolates to overseas isolates using MLST is limited by lack of sequence type data from neighbouring endemic countries. The ongoing outbreak of cholera in PNG highlights the many challenges faced in low-income countries when faced with new incursions of emerging infectious diseases. Given the socio-economic conditions and geography of PNG, it appears likely the disease will remain endemic.

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MALNUTRITION AND DIARRHEAL DISEASES IN A CASE CONTROL STUDY IN THE BRAZIL SITE

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About 53% (5.6 million) of the global <5yo deaths are associated with diarrheal diseases and malnutrition. The objective of this study was to identify the risk factors in children associated with malnutrition, diarrheal diseases, impaired gut function, vaccine response, impaired development and cognitive functions in case control epidemiological study in Northeast Brazil. The design of the case control was a prospective study of approximately 500 cases and 500 controls age and neighborhood matched controls, age 6-24 months, with follow-up studies at 1, 3, 6 and 12 months for the disease morbidity, microbiological, clinical, nutritional, gut function and cognitive function assessments. Cases will be defined as moderate to severe malnutrition, defined as WAZ <-2, and controls will be defined as WAZ >-1. Results: Up to 06Apr11 we had screened 163 and 160 (98%) mothers who signed the consent form had their children

eligible and none refused to participate in the study protocol. One child was lost to follow-up and one died. 44% (71/160) of the total enrolled were males, and 85 were cases and 75 were control children. The mean (SD) age at entry was 403 days (166.4). The proportion of breastfeeding at enrolment was 44% (37/85) for cases and 69% (52/75) for controls. A total of 178 stool samples were collected and 12 (6.7%) were from diarrheal stools samples. The mean (SD) WAZ z-score was -2.578 (1.028) and 0.064 (1.054) for cases and controls, respectively. A total of 1,414 microbiology tests have been performed. Enteroaggregative E. coli (EAEC) was significantly associated with cases (22/88; 25%) compared with controls (4/62; p=0.0147 by Fisher's exact test). The lactulose:mannitol ratio for the cases was normal in 60% (47/79) and 48% (33/69) for the control of the tests performed in the children enrolled so far. In conclusion, we documented striking differences in their nutritional z-scores and substantial disruption in intestinal barrier function. In addition, EAEC was significantly associated with malnourished children compared to non-malnourished paired controls.

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MODERATE-TO-SEVERE DIARRHEA AMONG CHILDREN LESS THAN FIVE YEARS OLD WITH HIV INFECTED MOTHERS IN RURAL WESTERN KENYA

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Diarrhea causes substantial morbidity and mortality among people living with HIV. Data on diarrhea among HIV-infected children are limited. We examined the outcome and etiology of moderate-to-severe diarrhea in Kenyan children <5 years old participating in the Global Enterics Multicenter Study (GEMS) who were HIV-infected (HIV+), HIV-uninfected, but potentially HIV-exposed (HIV-infected mother) (HIV-/+), and HIV-uninfected and HIV-unexposed (HIV-uninfected mother) (HIV-/-). Stool specimens were collected at enrollment. We abstracted HIV test results for enrolled children and their biological mothers. HIV infection was determined by PCR for children <18 months old, and by rapid antibody test for those >18 months. From Jan 25, 2010 to Feb 6, 2011, 206 (67%) of the 309 children with moderate-to-severe diarrhea had an HIV test at GEMS enrollment; 9 (4%) were HIV+, 45 (22%) HIV-/+, and 152 (74%) HIV-/-. Median age was 15, 11 and 13 months for the three groups, respectively. For HIV-infected mothers of HIV+ and HIV-/+ children respectively, the median CD4 count was 331 cells/μL and 451 cells/μL; 2 (22%) and 12 (27%) were on antiretroviral therapy. Five (56%) HIV+, 27 (60%) HIV-/+, and 115 (76%) HIV-/- children were currently breastfeeding. On enrollment, for HIV+, HIV-/+, and HIV-/- children respectively, 75%, 31%, and 27% were stunted (height-for-age z-score <-2), and 13%, 23%, and 10% were underweight (weight-for-age z-score <-2). Two (22%) HIV+, four (9%) HIV-/+, and 20 (13%) HIV-/- children were hospitalized for diarrhea. ETEC (33%), *Cryptosporidium* (25%), EPEC (22%), and astrovirus (14%), were more commonly found in stools from HIV+ children, than in stools from HIV-/+ and HIV-/- children (ETEC 16% and 19%; *Cryptosporidium*7% and 12%; EPEC 16% and 7%; astrovirus 0% and <1%). Death within 60 days of enrollment was more common among HIV-/+ children (4.4%) than among HIV-/- (0.7%) or HIV+ children (0%). These limited preliminary data suggest that being a child of an HIV-infected mother, regardless of the child's HIV status, adversely impacts outcome of diarrheal illness.

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RESISTIN-LIKE MOLECULE (RELM)α REGULATES TH17 CELL RESPONSES AND BACTERIAL INFECTION-INDUCED INTESTINAL INFLAMMATION

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RElsistin-Like Molecule (RELM)α belongs to a family of secreted mammalian proteins that have potential immunoregulatory functions. Following infection with the enteric pathogen *Citrobacter rodentium*, we observed significant increases in RELMα expression both locally in the colon and systemically in the serum. To test the role of RELMα in *Citrobacter* infection, we employed RELMα-/- mice. In comparison to wild-type mice, *Citrobacter*-infected RELMα-/- mice exhibited similar bacterial burdens but reduced infection-induced intestinal inflammation, characterized by decreased leukocyte recruitment to the infected colons and reduced immune cell activation. Further, infected RELMα-/- mice showed decreased expression of proinflammatory cytokine IL-17A. Supporting a proinflammatory function for RELMα, recombinant RELMα treatment of *Citrobacter*-infected mice exacerbated intestinal inflammation and IL-17A expression. To test if the mechanism by which RELMα promoted *Citrobacter*-induced intestinal inflammation was by inducing IL-17A expression, infected wild-type and IL-17A-/- mice were treated with recombinant RELMα. In contrast to RELMα-treated wild-type mice, RELMα treatment of IL-17A-/- mice did not exacerbate *Citrobacter*-induced inflammation. Together, these data support a pathogenic role for RELMα in inducing inflammation at mucosal surfaces, in part through promoting IL-17A expression.

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HIGH QUINOLONE RESISTANCE IN *CAMPYLOBACTER SPP.* ISOLATES FROM DIARRHEA AND HEALTHY CONTROL CASES FROM PERUVIAN CHILDREN UNDER TWO YEARS OF AGE

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Quinolones are considered as the drugs of choice for campylobacteriosis. The aim of this study was to determine the quinolone resistance in *Campylobacter spp.* isolated from Peruvian children under 2 years of age, with and without diarrhea. 96 *Campylobacter spp.* isolated from a cohort study in Lima, Peru were tested. The isolates were identified using standard procedures; a multiplex PCR was performed using primers previously described to identify *Campylobacter* species. The evaluation of susceptibility was performed by agar diffusion disk method to 4 antimicrobial drugs: ciprofloxacin (CIP), tetracycline (TE), erythromycin (E), and azithromycin (AZM). A DNA fragment of 410 bp containing the quinolone resistance-determining region of the *gyrA* gene for 20 *C. jejuni* strains was obtained by PCR and the DNA was sequenced. From the 96 strains of *Campylobacter spp.,* 44% (42/96) were from diarrhea and 56% (54/96) were from healthy control cases; 55% (53 strains) were *C. jejuni*, and 45% (43 strains) were *C. coli*. 89% (85/96) were CIP resistant (87% C. *jejuni,* and 91% *C. coli);* 55% (45/82) were TE resistant (60% C. *jejuni,* and 49% *C. coli);* 13% (12/96) were E resistant (4% *C. jejuni* both from diarrhea cases, and 23% *C. coli*), and 13% (11/84) AZM resistant (2% *C. jejuni,* one from a diarrhea case, and 26% *C. coli*). In 20 *C. jejuni* strains, the *gyrA* gene was analyzed presenting a single change in the Thr-86 of GyrA to Ile, one strain presented the change from Thr-86 of

GyrA to Ala; in this group, 4 CIP susceptible isolates presented the amino acid substitution Thr-86 to Ile, and one Thr-86 to Ala**.** Our results indicate that *Campylobacter* resistance to quinolones is high in Peruvian children. Mutation in the Thr-86 of the GyrA protein is a frequent mechanism associated with the acquisition of resistance to quinolones in clinical isolates of *C. jejuni*.

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DETECTION OF SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI* (STEC) IN HEALTHY CATTLE AND PIGS IN LIMA, PERU

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Shiga toxin-producing *Escherichia coli* (STEC), has emerged as a group of foodborne pathogens that can cause severe human disease, such as hemolytic uremic syndrome. STEC can be found in the fecal flora of a wide variety of animals including cattle and pigs and they are the most important animal species in terms of human infection. The aim of this study was to determine the prevalence of STEC in cattle and pigs as a possible reservoir of STEC in Lima, Peru. One hundred fourteen cattles and 112 pigs from 11 and 3 farms in Lima, Peru, respectively, were studied. All animals in this study were healthy and without treatment with antibiotics. Stool samples were obtained with a rectal swab. Five *E. coli* colonies per animal were studied by a multiplex real-time PCR to identify Shiga toxin-producing (*stx1, stx2, eaeA*), Enterotoxigenic (*lt, st*), Enteropathogenic (*eaeA* alone), Enteroinvasive (*IpaH*), Enteroaggregative (*AggR*), and Diffusely Adherent *E. coli* (*daaD*). One colony *stx1* and/or *stx2*-positive from each strain were classified as Shiga-toxinogenic using an enzymatic immunoassay Shiga-toxin test and were tested for O157 serogroup using an *E. coli* antisera kit. All STEC-positive samples were also cultured on MacConkey-sorbitol agar. STEC was isolated from 16 out of 114 bovines cultures (14%). STEC was not isolated from pigs. The *stx1* gene was found in all isolates, 11 of which also carried *eaeA* genes (69%). Only one sample was found to have both Shiga-toxin genes (*stx1* and *stx2*). Thirteen *stx*-positive strains were classified as Shiga-toxinogenic (81%). Only two STEC strains were positive for O157 serogroup (13%). Seven STEC-positive stool samples were sorbitol negative (44%). Additionally, Enteropathogenic *E. coli* were detected more frequent in cattle (18%, 20/114) than pigs (5%, 6/112), $p<0.01$. This study represents, to our knowledge, the first survey on the prevalence of STEC in farms animals in Lima, Peru using molecular methods. Further studies are needed to evaluate the participation of cattle and pigs as STEC reservoir at local farms which could have serious consequences for public health.

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ANALYSIS OF *SALMONELLA ENTERICA* SEROTYPE TYPHI GENE EXPRESSION IN THE BLOOD OF PATIENTS WITH TYPHOID FEVER IN BANGLADESH

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Salmonella enterica serovar Typhi is the cause of typhoid fever. *S.* Typhi is a human-restricted pathogen, but few data exist on *S*. Typhi gene expression in humans. We applied an RNA capture and amplification technique, Selective Capture of Transcribed Sequences (SCOTS), and microarray hybridization technology to identify *S.* Typhi transcripts expressed in the blood of five humans infected with *S.* Typhi in Bangladesh. In total, we identified 2046 *S.* Typhi genes (44% of the *S.* Typhi genome) in human blood; we detected 912 genes in all 5 patients and 1100 genes were detected in 4 or more patients. Identified transcripts were associated with the virulence-associated PhoP regulon; *Salmonella* pathogenicity islands; the use of alternative carbon and energy sources; synthesis and transport of iron, thiamine, and biotin; and resistance to antimicrobial peptides and oxidative stress. The most highly represented group were genes currently annotated to encode hypothetical proteins or proteins designated as unknown or unclassified. Of the 2046 detected genes, 1320 (29% of *S*. Typhi ORFeome) had significantly different levels of transcriptional expression in human blood compared to *in vitro* cultures; 141 genes were significantly different in all 5 patients, and 331 in at least 4 patients. These genes were largely of unknown function, or involved in energy metabolism, transport and binding, cell envelop or cellular processes and pathogenesis. We confirmed increased expression of a subset of identified transcripts using quantitative-PCR. This is the first characterization of the bacterial transcriptional profile in the blood of humans with typhoid fever, a major global pathogen. Our results suggest that *S*. Typhi in the blood of infected humans express genes involved in intra-cellular survival and alternative energy sources, as well as many genes whose function is currently unknown.

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INCREASED RISKS OF CLINICAL MALARIA AND *PLASMODIUM* PARASITEMIA IN HIV-1 INFECTED AGRICULTURAL WORKERS AND DEPENDENTS: THE KERICHO COHORT STUDY

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Limited data describing the malaria-HIV interaction are available from long-term, prospective studies with serial parasitemia and HIV evaluations. We evaluated relationships between HIV and *Plasmodium* parasitemia and clinical malaria in adults participating in the Kericho Cohort Study, a

36-month, prospective study with biannual visits including medical history and clinical evaluations, HIV testing, and clinical microscopy. Kaplan Meier survival curves were created and multivariable regression analyses were used to estimate odds ratios (OR) for baseline prevalent cases and hazards ratios (HR) for follow-up incident cases with 95% confidence intervals (CI) between HIV and two malaria outcomes: 1. *Plasmodium* parasitemia, and 2. combined parasitemia and/or history of clinical malaria. 2801 volunteers (mean age+/-SD=30.5+/-8.5 years, women=38.4%) enrolled with baseline HIV prevalence=14.3% and baseline combined parasitemia/clinical malaria prevalence=33.2%. 96% of parasitemia cases were *P. falciparum*. More HIV infected compared to non-infected participants enrolled had baseline parasitemia (6.0% vs. 3.5%, p=0.02) and parasitemia/clinical malaria (40.0% vs. 31.0%, p<0.01) corresponding to ORs of 1.73 (95% CI=1.04-2.80) and 1.53 (95% CI=1.22-1.91), respectively, which were robust after controlling for age and gender: 2.00 (95% CI=1.24-3.24) and 1.51 (95% CI=1.21-1.88). In prevalent and incident parasitemia cases, adults with HIV had higher 4th quartile proportions of parasitemia: 54.0% vs. 28.0% (p=0.03) and 38.0% vs. 18.0% (p=0.02), respectively. Survival times to parasitemia and parasitemia/clinical malaria were significantly different given baseline HIV status (log rank test p<0.01 for both). Adults with baseline HIV were at increased risks of incident parasitemia and parasitemia/clinical malaria: HR=1.69 (95% CI=1.29-2.21) and HR=1.41 (95% CI=1.19-1.69), respectively, which were robust after adjusting for age, gender, and incident HIV: HR=1.78 (95% CI=1.35-2.35) and HR=1.37 (95% CI=1.15-1.64). We conclude HIV-1 infected adults were at increased risks for *Plasmodium* parasitemia and combined parasitemia or history of clinical malaria. Adults with HIV and malaria had higher parasitemia levels with advanced HIV status.

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TREATMENT OF LYMPHATIC FILARIASIS (LF) IN HIV/LF CO-INFECTED INDIVIDUALS IN CHENNAI, INDIA

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The disease course of human immunodeficiency virus (HIV) is often altered by existing or newly acquired coincident infections. Treatment or prevention of these concomitant infections often improves the quality and duration of life of HIV-infected persons. The impact of helminth infections (particularly tissue invasive infections) on HIV infection is less clear. To assess the influence of pre-existing *Wuchereria bancrofti* infection on HIV progression, we performed a case-controlled treatment study of HIV positive individuals with (Fil+) or without (Fil-) *W. bancrofti* infection. We used the standard recommended regimen of albendazole/diethylcarbamazine (DEC/Alb) that is the mainstay of mass drug administration in India to treat 28 HIV+/Fil+ and 52 HIV+/Fil- subjects (1:2 matched for age, gender, CD4 count, initial HIV viral load and antiretroviral medication). We assessed all subjects at baseline and at 1, 3, 6 and 12 months following drug administration. At baseline, there was no difference in mean CD4 counts (370/ml [95%CI 271-503] vs. 468/ml [95%CI 396-553]; p=0.17) or viral loads (6202 copies/ml [95%CI 1702-13286] vs. 4403 copies/ml [95%CI 2154-9000]; p=0.90) between the HIV/FIL+ and HIV/Fil- groups. Following DEC/Alb, there were no differences noted in clinical outcomes between the groups. There also was no difference in the HIV viral load at 12 months (5064 copies/ml [95%CI 1497-17127] vs. 3880 copies/ml [95%CI 1860-8094]; p=0.78) between the two groups. Furthermore, there were no significant differences found in either the change in viral load at 1, 3, or 6 months (p>0.5 for all comparisons) or in the CD4 count at 3, 6, or 12 months (p>0.5 for all comparisons). CD4 counts between the two groups were also similar at 1 year (452/ml [95%CI 361-565] for the HIV/FIL+ group and 556/ml [95%CI 375-571] for the HIV/FIL- group). Although our study was limited by the numbers of study participants—the prevalence of lymphatic filariasis having diminished in South India - we were unable to find a significant effect of *W. bancrofti* infection or its treatment on HIV clinical course or surrogate markers of HIV disease progression.

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EXTENDED COTRIMOXAZOLE PROPHYLAXIS AND MORBIDITY IN FORMULA-FED, HIV-EXPOSED, UNINFECTED INFANTS, BOTSWANA

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High mortality has been observed among African HIV-exposed uninfected (HIV-EU) infants, particularly if formula-fed. Cotrimoxazole may help mitigate this vulnerability, but could exacerbate antiretroviral-associated hematologic toxicity. To assess the impact of extended cotrimoxazole prophylaxis (CTX), we enrolled sequential HIV-infected mothers and their infants in one city and one village in Botswana for a pilot study of daily cotrimoxazole (<5 kg: 100mg sulfamethoxazole, 20mg trimethoprim; ≥5 kg 200mg sulfamethoxazole, 40mg trimethoprim) from 1 to 6 months of age. Data were pooled with data from the Mashi PMTCT trial conducted in the same communities. Full blood count and HIV DNA PCR was measured in both studies at 1, 3-4, and 6-7 months. Mothers received antenatal zidovudine or combination antiretroviral therapy, and infants received 1 month of zidovudine. We compared rates of severe anemia, severe neutropenia (Grade 3 or 4, Division of AIDS, 2004), and occurrence of hospitalization or death due to any cause between CTX groups. We used exact statistical methods and restricted analysis to formula-fed, HIV-EU infants alive at 1 month. Of 711 infants with scheduled hematology measurements, severe anemia was detected in 2 infants (1.1%, 95%CI 0.1-3.8%) receiving CTX and 13 infants (2.5%, 95%CI 1.3-4.2) not receiving CTX (P=0.38). Severe neutropenia was diagnosed in 11 infants (5.8%, 95%CI 2.9-10.1%) receiving CTX and 27 infants (5.2%, 95%CI 3.4-7.5%) not receiving CTX (P=0.71). Of 918 infants, the composite endpoint of hospitalization or death occurred in 10 infants (5.1%, 95%CI 2.5-9.2%) receiving CTX and 67 infants (9.3%, 95%CI 7.3-11.6%) not receiving CTX (P=0.08). Multivariate analysis adjusting for baseline differences in maternal CD4 count, birth characteristics, and socioeconomic status did not modify the univariate findings. Risk of severe anemia and neutropenia in formula-feeding HIV-EU is low and does not appear to be increased with CTX. Trend towards decreased risk of hospitalization or death with CTX deserves evaluation in larger, randomized studies.

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PREDICTORS OF CHILDBEARING INTENTIONS AMONG CLIENTS ATTENDING TASO JINJA, UGANDA

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Although the desire of HIV-infected persons to have children has important implications for the transmission of the virus to their sexual partners and newborns, HIV positive status has not significantly influenced childbearing in Uganda. This study assessed the reproductive intentions among HIV positive clients attending "The AIDS Support Organisation" (TASO) Jinja centre in eastern Uganda. Five hundred twenty eight

consenting clients aged 15-49 years were enrolled at static centre clinics, outreach posts and community drug distribution points from May to July 2010. About a third (183/528) had plans to bear children. The mean age of those who planned to have more children was 33 years [95%CI: 31.7-34.3]. Men were more likely to plan to have additional children than women (p=0.016), although they were older 38 years [95%CI: 35.9-40.0] than females 30.6 years [95%CI: 29.1-32.1]. Thirty (7.7 %) women were pregnant at the time of interview. Having five children or less was positively associated with need for more (p<0.001) regardless of gender. Being employed (p=0.016), having one sexual partner (p=0.05) and being a male on antiretroviral drugs for 6 or more months was associated with being less likely to desire more children (p=0.032). Clients had a general belief that bearing children strengthens the marital relationship and cements commitment from male partners, yet the prevention of mother to child transmission of HIV programme created a window of opportunity to bear HIV-negative children. In conclusion, a substantial proportion of HIV positive clients had the desire for more children to fulfil their marital obligation and to cement commitment from partners. The predictors of childbearing while living with HIV included: gender, number of children ever had, employment, number of sexual partners, possession of biological children and current use of ARVs. There is need to adjust the model of delivery of HIV services so that socially and culturally engrained client concerns are also addressed.

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EFFECT OF DAILY TRIMETHOPRIM-SULFAMETHOXAZOLE PROPHYLAXIS ON THE RISK OF GAMETOCYTEMIA IN UGANDAN CHILDREN

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The use of sulfadoxine-pyrimethamine has been associated with an increased risk of gametocytes, the transmissible stage of malaria. Daily prophylaxis with another antifolate combination, trimethoprim-sulfamethoxazole (TS), has been shown to reduce the incidence of malaria; however data on the impact of TS on gametocytemia is limited. A total of 100 HIV-unexposed, 203 HIV-exposed (born to HIV-infected mothers) and 48 HIV-infected children were enrolled between 1.5-12 months of age and followed until 36 months of age. All HIV-infected and breastfeeding HIV-unexposed children were taking TS prophylaxis. HIV-exposed children were randomized to stop or continue TS after breastfeeding after excluding HIV and at 2 years of age. Blood smears were performed when children presented with fever, during standardized 28-day malaria follow-up, and at routine monthly visits. Gametocytes were diagnosed by microscopy and reported as present or absent. We compared the monthly risk of gametocytemia in children prescribed TS and not prescribed TS, stratified by visit type, using generalized estimating equations adjusting for residence, age, and assigned antimalarial treatment group. There were 4000 complete months of observation with at least one blood smear done where TS was prescribed and 5061 months where TS was not prescribed. Although the use of TS was associated with a lower monthly risk of malaria (19% vs. 43%, p<0.001), there was no significant difference in the monthly risk of any gametocytemia (4.4% vs. 4.9%, adjusted RR=1.26, p=0.15). TS was associated with a significant higher monthly risk of gametocytemia during months where malaria was diagnosed (7.7% vs. 4.8%, aRR=1.74, p=0.005) and during malaria follow-up (8.0% vs. 5.4%, aRR=1.74, p=0.001). There was a trend towards a higher risk during monthly routine visits (2.0% vs. 1.7%, aRR=1.55, p=0.09). In this cohort, daily TS prophylaxis reduced the risk of malaria but increased the risk of gametocytemia when malaria occurred, potentially having an adverse effect on malaria transmission.

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PREDICTORS OF SWITCHING ANTIRETROVIRAL REGIMEN AMONG CLIENTS ATTENDING TASO JINJA, UGANDA

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The Uganda Ministry of Health recommends AZT/3TC or TDF/3TC or TDF/FTC or D4T/3TC + NVP or EFV as first line antiretroviral drug combinations for treatment of HIV. Information from clinical practice indicates that HIV positive clients are switched on these drugs without following guidelines. This policy evaluation assessed the predictors of switching antiretroviral drug combinations in both facility-based and home-based intervention models in August-December 2008 among 326 HIV positive clients on antiretroviral drugs who were attending The AIDS Support Organization (TASO) Jinja, Uganda. The outcome measure was switching antiretroviral regimen. Thirty one (11.4%) clients were in WHO clinical stage 4, stage 3 were 115(35.3%), 126(38.7%) in stage 2 and 1(0.3%) in stage1. One hundred fifty (46.0%) received drugs under the home-based model. The proportion of clients on first line ART was 325(99.7%). One hundred forty two (43.6%) were started on AZT, 3TC, NVP; 132(40.4%) on d4T, 3TC, NVP; 26(8.0%) on d4T, 3TC, EFV; 24(7.4%) on AZT, 3TC, EFV; and 2(0.6%) on TDF, 3TC, NVP. Some 60.3% developed side effects [peripheral neuropathy 93(28.5%), anaemia 27(8.3%), skin rash 10(3.1%), lipodystrophy 5(1.5%)] following ART initiation. Sixty three (19.3%) had their first antiretroviral drug combinations switched either by clinicians 48(14.3%), pharmacy technicians 1(0.3%), nurses 3(0.9%) or field officers 5(1.8%). Twenty two (6.7%) had their antiretroviral drugs switched for the second time, 7(2.1%) changed for the third time, and 3(0.9%) changed for the fourth time. All switches were within the first line ART combinations. The antiretroviral drug combination that the client was started on (d4T/3TC/NVP) (p=0.002), development of side effects (p<0.001) and lack of medicine companion or treatment supporter (p=0.039) were associated with switching of antiretroviral drugs. In order to control the switching of drugs, ART team members should be involved in decision making.

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CORRESPONDENCE OF THE TEN QUESTIONS QUESTIONNAIRE (TQQ) TO DEVELOPMENTAL MEASURES FOR RURAL UGANDAN PRESCHOOL CHILDREN WITH HIV

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The Ten Questions Questionnaire (TQQ) is widely used for screening neurodisabilities in children. It has been adapted globally in numerous low-resource settings for field research, but has not been extensively validated in Africa, especially in rural settings. The objective is to evaluate the correspondence of the individual items of the TQQ to various developmental and cognitive assessment domains for preschool Ugandan children with HIV**.** 113 Ugandan children with HIV between 2 and 6 years of age (40% on HAART medication) in rural Kayunga District were screened with the TQQ administered to the principal caregiver as part of a home evaluation. The next day they were screened with Mullen Early Childhood Scales, Color Object Association Test (COAT) of memory, Early Childhood Vigilance Test (ECVT) of attention, Achenbach Child Behavior Checklist (CBCL) given to the principal caregiver. For each TQQ item, children who screened positive for a neurodisability problem (15 to 20 %) were compared to those who screened negative. Comparisons were made using an ANCOVA with age, gender, SES, HAART status, and arm circumference in proportion to height as covariates.The TQQ speech difficulties item was significantly related to Mullen Expressive Language (P=.003), Receptive Language (P=.004), Visual Reception (P =.04), Fine Motor (P=.02). These relationships were mediated in part by

viral load and CD8 activation measures at time of assessment (regression analysis). The TQQ item of difficulty walking was significantly related to Mullen Gross Motor (*P*=.0001) and Fine Motor (*P*=.015). TQQ hearing difficulties was related to poorer COAT memory performance (P=.01). TQQ Learning and Listens to Instructions were significantly related to the Mullen Expressive Language measure. Only certain items of the TQQ had good correspondence to a more thorough measure of development and cognition. Speech problems and walking difficulties especially seemed to be good screening items for Mullen developmental assessment outcomes in younger rural Ugandan children with HIV.

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IDENTIFICATION AND FUNCTIONAL VALIDATION OF THE NOVEL ANTIMALARIAL RESISTANCE LOCUS PF10_0355 IN *PLASMODIUM FALCIPARUM*

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Malaria's ability to rapidly adapt to new drugs has allowed it to remain one of the most devastating infectious diseases of humans. Understanding and tracking the genetic basis of these adaptations is critical to the success of therapeutic and intervention strategies. We developed a high-density genotyping array covering more than seventeen thousand single nucleotide polymorphisms (SNPs) across the *Plasmodium falciparum* genome (~1 SNP/kb), and applied it to fifty culture-adapted malaria parasites from three continents. We also created a platform for high throughput characterization of drug-sensitivity phenotypes and performed genome-wide association studies (GWAS) for resistance to thirteen antimalarials. In addition to detecting the known chloroquine resistance locus pfcrt, we discovered a number of novel loci associated with resistance to amodiaquine, artemisinin, atovaquone and halofantrine. We next followed up a novel halofantrine resistance-associated locus, PF10_0355, uncovered by this approach. Functional analysis revealed that overexpression of PF10_0355 confers decreased sensitivity to halofantrine, mefloquine and lumefantrine but does not alter parasite susceptibility to other, structurally unrelated antimalarials. This effect was restricted to the later asexual stages, when PF10_0355 is expressed and is present on the merozoite surface. Knockout of the PF10_0355 gene increased parasite sensitivity to halofantrine, mefloquine and lumefantrine, but not to unrelated antimalarials, further suggesting that parasite resistance is mediated by copy number variation at this locus. Our results demonstrate the power of genome-wide approaches to identify drug resistance loci and point to PF10_0355 as a novel mediator of drug resistance in the malaria parasite.

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A SYSTEMATIC SCREENING APPROACH IDENTIFIES AN ERYTHROCYTE RECEPTOR FOR PFRH5 THAT IS ESSENTIAL FOR INVASION

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Erythrocyte invasion is essential for *Plasmodium falciparum* survival and pathogenesis. Invasion is catalyzed by multiple interactions between parasite ligands and their receptors on human erythrocytes, with most of these interactions thought to have overlapping and redundant roles. However, although multiple invasion ligands are known, in very few cases have their cognate erythrocyte receptors been identified, in part because cell surface protein-protein interactions are often of very low affinity, making them hard to identify using standard biochemical approaches. We have applied AVEXIS, a systematic protein interaction screening approach that is designed to detect low affinity extracellular interactions, to identify an erythrocyte receptor for PfRh5. Members of the reticulocyte-binding homology (PfRh) family have been implicated in invasion, but only *PfRh5* has been shown to be essential for parasite growth. We showed that the PfRh5 receptor is essential for parasite entry in every *P. falciparum* strain tested to date, as invasion *in vitro* is potently blocked in all parasite strains tested by soluble receptor ectodomains and by receptor-specific monoclonal antibodies. Furthermore, a naturally occurring polymorphism in the receptor, with reduced affinity for PfRh5, was associated with lower invasion efficiencies. The application of the AVEXIS technology involves production of full-length recombinant *P. falciparum* proteins, and we have expanded the expression approach from PfRh5 to include more than 30 merozoite proteins, including some of more than 200kDa in size. This approach will have many applications for immunoepidemiology, vaccine development, and the understanding of *P. falciparum* protein function.

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APTAMER-BASED DISCOVERY OF A CONSERVED MALARIAL RED CELL PROTEIN

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The human malaria parasite, *Plasmodium falciparum*, invades erythrocytes and modifies the host cell surface with a complex and poorly defined array of proteins that undergo rapid antigenic variation. These proteins also mediate the selective uptake of serum nutrients, an essential strain-independent function that suggests some molecules on the variable parasitized red cell surface are conserved. To explore this possibility, we developed a DNA aptamer selection scheme to probe for sites common between ten geographically distinct parasite lines. Our scheme evolved a single aptamer from a large combinatorial library. The aptamer binds infected red cells but not uninfected cells and efficiently kills blood stage parasites *in vitro* in a dose-dependent and sequence-specific manner. Growth inhibition is not due to blockade of parasite nutrient influx. These data highlight the importance of host cell modifications to the malaria parasite's survival and support further development of the selected aptamer as a novel antimalarial agent.

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THE SYNERGISTIC EFFECT OF HSP90 INHIBITORS AND CHLOROQUINE AGAINST MALARIA MAY BE EXPLAINED BY PFHSP90-PFCRT INTERACTION

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To successfully overcome heat shock stress, the functional stability of proteins requires buffering offered by Hsp90. Hsp90 appears to be essential for cell cycle progression in various models and we postulate plays a role in the expression of drug resistance determinants. Recent crystal structure data supports that malaria Hsp90 has unique properties amenable to small molecule inhibition. Dual targeting of PfHsp90 and one of its client proteins may provide an effective strategy for the identification of synergistic drug combinations with the potential to circumvent drug resistance. We and other groups have previously shown that Hsp90 inhibitors synergize with conventional antimicrobials when used in combination. The objectives of this study are: 1) to test the synergistic potential of a purine analog to reverse antimalarial resistance *in vitro*; 2) to determine if the synergistic activity of PfHsp90 inhibitors with chloroquine can be explained by a protein-protein interaction between PfHsp90 and the chloroquine transporter PfCrt; 3) to determine the full interactome of PfHsp90 using co-immunoprecipitation and mass spectrometry; 4) to test synergistic drug combinations in a mouse model of malaria. Several methods were undertaken: 1) antimalarial activity of PfHsp90 inhibitors was determined using a cell-based assay; 2) association of PfHsp90 with its client proteins and the chloroquine transporter PfCrt was evaluated using co-immunoprecipitation coupled with mass spectroscopy analysis; 3) parasite stage-specific growth arrest in relation to heat shock stress was studied in the presence and absence of PfHsp90 inibition; and 4) PfHsp90 inhibitor combinations with chloroquine were tested in the *P. berghei* murine model of malaria. A purine analog was able to reverse resistance to CQ in *Plasmodium falciparum in vitro*. Co-immunoprecipitation revealed the direct association of PfHsp90 with the chloroquine transporter PfCrt. Combination of the purine analog and CQ demonstrated potent combination effects in the *P.berghei* mouse model of malaria. In conclusion, we describe a drug combination that reverses chloroquine resistance *in vitro* and demonstrates synergy *in vivo*. This phenotype may be explained by a direct association of PfHsp90 with PfCrt. Further characterization of PfHsp90 and its interactome for dual targeting affords the possibility of developing several adjunctive drug therapies that may reverse antimalarial resistance.

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DEFINING MINIMAL REACTIVE EPITOPES ON THE SURFACE OF *PLASMODIUM VIVAX* DUFFY BINDING PROTEIN REACTIVE WITH NEUTRALIZING MONOCLONAL ANTIBODIES

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The *Plasmodium vivax* Duffy Binding protein (DBP) is a vital ligand for blood-stage development making the molecule an attractive vaccine candidate for inclusion in a vaccine designed to protect against *vivax* malaria. Similar to other blood-stage vaccine candidates, DBP allelic variation eliciting a strain-specific immunity may be a major challenge for development of a broadly effective vaccine against *vivax* malaria. To understand the nature and location of epitopes that can be the target of neutralizing anti-DBP inhibition, we generated a panel of monoclonal antibodies to DBP and functionally analysed their reactivity to a panel of allelic variants. Quantitative analysis by ELISA determined that some monoclonals reacted strongly with epitopes conserved on all DBP variants tested, while reactivity of other monoclonals was allele-specific. Qualitative analysis characterized monoclonal anti-DBP functional inhibition, using an *in vitro* erythrocyte-binding inhibition assay. There was not a consistent correlation between the end point titers and functional inhibition, but some monoclonals were broadly inhibitory while inhibition of others varied significantly by target allele. Using phage display, we mapped the epitopes of the monoclonal antibodies to primarily subdomain III. The minimal reactive epitopes were mapped using a random gene fragment phage library of DBPII. Our data demonstrate a potential for vaccine-elicited immunization to target conserved epitopes but optimization of DBP immunogenicity may be needed for protection against diverse *P. vivax* strains. Ultimately information derived from these studies will contribute to the assessment of this antigen for inclusion in a vaccine designed to protect against disease caused by *vivax* malaria.

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COLONIZATION OF *PHLEBOTOMUS PAPATASI* CHANGES THE EFFECT OF PRE-IMMUNIZATION WITH SALIVA FROM LACK OF PROTECTION TOWARDS PROTECTION AGAINST EXPERIMENTAL CHALLENGE WITH *LEISHMANIA MAJOR* AND SALIVA

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Sand fly saliva has been postulated as potential vaccines or as a vaccine component within multi component vaccine against leishmaniasis. While pre-immunization of mice with salivary gland homogenate (SGH) of long-term colonized (F5 and beyond) female *Phlebotomus papatasi* induced protection against *Leishmania major* co-inoculated with the same type of SGH, pre-immunization of mice with SGH of recently colonized (F2 and F3) female *P. papatasi* did not confer protection against *L. major* co-inoculated with the same type of SGH. Interestingly, our data showed for the first time that a shift from exacerbation to protection occurs at the fourth generation (F4) during the colonization process in *P. papatasi*. Hence, for the development of a sand fly saliva-based vaccine, inferences based on long-term colonized populations of sand flies should be met with caution as colonization of *P. papatasi* appears to modulate the outcome of *L. major* infection from exacerbation to protection.

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EXPERIMENTAL TRANSMISSION OF KARSHI (MAMMALIAN TICK-BORNE FLAVIVIRUS GROUP) VIRUS BY *ORNITHODOROS* TICKS (*ACARI: ARGASIDAE*) MORE THAN 2,000 DAYS AFTER INITIAL VIRUS EXPOSURE

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Members of the mammalian tick-borne flavivirus group are believed to be maintained in nature by infected, overwintering, nymphal ixodid ticks infecting field rodents in the spring that, in turn, infect larval ixodid ticks. However, because of the short period of viremia in rodents, this would require exquisite timing to serve as a reliable maintenance mechanism. To attempt to examine an alternative mechanism involving soft ticks that would allow for the long-term maintenance of these viruses, we followed *Ornithodoros parkeri*, *O. sonrai*, and *O. tartakovskyi* for >2,000 days after they had been exposed to Karshi virus. Ticks were exposed to

Karshi virus either by allowing them to feed on viremic suckling mice or by intracoelomic inoculation. The ticks remained efficient vectors of this virus to suckling mice when tested >2,000 d after their initial exposure to virus, whether initially exposed orally or by inoculation. The ability of these ticks to survive and remain infectious for many years indicates that they may be involved in the long-term maintenance of this group of viruses.

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EVALUATION OF REPLICATION OF YELLOW FEVER 17D/ DENGUE CHIMERIC VIRUSES IN HARD TICKS (*IXODIDAE*)

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Arthropod-borne viruses (arboviruses) of the Flaviviridae family (e.g., TBE, Yellow fewer, dengue, West Nile viruses) are important pathogens causing severe diseases in humans worldwide. Biological transmission of arthropod-borne flaviviruses involves tree major players: the virus, a competent vector and a susceptible vertebrate host. Blood-feeding arthropods (mosquitoes, ticks) are important vectors of flaviviruses. To determine if an arthropod is a competent vector of a virus, two main conditions must be fulfilled: (1) the virus must survive and replicate in arthropod cells and (2) the arthropod must be able to transmit the virus efficiently to a host. In an effort to develop a vaccine against mosquito-borne dengue viruses, a tetravalent vaccine candidate has been generated based on chimeric YF17D/DEN 1-4 viruses (CYD1-4). In order to determine if the tropism of the chimeric viruses changed compared to YFV-17D and parental DEN viruses, replication of CYD1-4 in ticks was assessed. CYD1-4 and parental DENV1-4 and YF17D viruses were inoculated parenterally into females of two hard tick species, *Ixodes ricinus* and *Rhipicephalus appendiculatus*. The persistence and replication of the viruses in ticks were assessed at various time points post-inoculation by plaque titration and qRT-PCR. Tick-borne encephalitis virus (TBEV) was used as positive control. Transmission potential of the viruses from infected to non-infected ticks was tested in the co-feeding model on laboratory mice. No replication of parental DENV1-4 was detected in ticks, whereas YF17D replicated at low level. Persistence of CYD1-4 was observed in *Ixodes ricinus* at early time points post-inoculation, however, at titres at least 100-fold lower than in TBEV-infected ticks. While TBEV persists in ticks, no CYD virus amplification was observed and viral clearance was observed by 44 days post-inoculation, except for some CYD2-infected *I. ricinus,* with a tendency to decline. Importantly, and in contrast to TBEV where about 70% of *I. ricinus* nymphs acquired infection by co-feeding with infected tick females, no co-feeding transmission of CYD2 was detected. In conclusion, considering the low/absent viremia after vaccination of humans with CYD vaccine, virus clearance in ticks, and absence of co-feeding transmission, it is highly unlikely that their dissemination by ticks could occur.

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AGE STRUCTURE OF HOST SEEKING DERMACENTOR VARIABILIS ON MARTHA'S VINEYARD, MASSACHUSETTS

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Martha's Vineyard, Massachusetts (MV), contains numerous stable natural foci of transmission for the agent of Type A tularemia. The yearly prevalence of infection in host seeking Dermacentor variabilis (dog ticks) there ranges from 1-5% (median 2.5) over the last decade, to our knowledge the only site ever reported with such stable transmission. We seek to determine the biological basis for such stability. Classical studies by Carroll Smith and colleagues on MV during the 1940s suggested that adult dog tick populations comprise two cohorts, the major one typically from nymphs that fed and molted within the season, and an early small one derived from nymphs that molted late in the previous season and immediately went into diapause to overwinter. Describing the age distribution of host seeking dog ticks has implications for interseasonal maintenance of an agent that tends to kill the vertebrate host. Because hard ticks take only one bloodmeal during each stage, and use energy reserves during host seeking, quantifying lipid reserves within a tick should provide an index of age. Accordingly, we stained lipids within malpighian tubules with Sudan red and subjectively scored the amount of lipids by microscopy, an assay previously used to analyze the age distribution of Ixodes ricinus. Surprisingly, a great diversity in the amount of lipids was detected in ticks collected during April, when they first start to seek hosts. Indices ranged from 1 to 4 with median of 2.5, with a normal distribution. If Smith's accepted phenology is correct, indices should reflect a great prevalence of high lipid reserve due to inactivity during winter. Instead, it may be that ticks failing to find hosts during the previous summer may have a prolonged life, overwintering and seeking hosts again the following year. We conclude that the phenology of the dog tick needs to be redescribed, particularly their population age structure, to identify factors that contribute to the stability of the transmission cycle of the agent of tularemia on MV.

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QUANTITATIVE PCR FOR DETECTION OF *BABESIA MICROTI*

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Babesia microti, the primary cause of human babesiosis, is transmitted by Ixodes scapularis ticks and through blood transfusion. Most infected people experience a viral-like illness that resolves without complication but those who are immunocompromised may develop a fulminant illness leading to a prolonged relapsing illness or death. Laboratory assays are needed for human diagnosis, epidemiologic tick, mouse, and human surveillance, and screening of blood donors to prevent transfusion transmitted babesiosis. Although polymerase chain reaction (PCR) is the best diagnostic assay for rapid confirmation of *B. microti* infection, current PCR assays lack the sensitivity and specificity for accurate detection. Accordingly, we developed a *B. microti* quantitative PCR primer and probe set targeting the 18S rRNA gene of *B. microti*. Using *B. microti*-infected SCID mice to develop a standard curve for comparison of parasitemia and DNA detection, we found that our Babesia qPCR could detect a minimum of 1 parasite per microliter of blood. Of 10 laboratory derived nymphal ticks that were generated from feeding on *Babesia* infected mice, *B. microti* DNA was detected in two ticks using a standard *B. microti* PCR assay and nine ticks using the *B. microti* qPCR assay. Neither assay amplified DNA in 20 uninfected tick samples. *B. microti*-infected mouse blood was assayed using both protocols. Of three samples tested, two were positive by qPCR and only one by PCR. Blood from three uninfected mice tested negative by both assays. We then tested the qPCR in people experiencing *B. microti* infection that was confirmed with blood smear or antibody serocoversion. *B. microti* DNA was detected in the blood of all 28 *B. microti*-infected patients and none of 15 patients without *B. microti* infection. All qPCR positive samples were confirmed as *B. microti* through sequencing. In sum, we have developed a highly sensitive and specific *B. microti* qPCR assay that is superior to the conventional *B. microti* PCR.

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LINKING ACAROLOGICAL RISK AND LYME DISEASE INCIDENCE IN THE USA

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Lyme disease is a zoonosis caused by *Borrelia burgdorferi* and transmitted in the eastern U.S, by the black legged tick Ixodes scapularis. Humans are incidental hosts, acquiring the pathogen through the bites of infected ticks. Acarological risk - the density of infected *Ixodes scapularis* nymphs, is often used as measure of human risk of infection, but how this quantity correlates with human incidence is not well-known. To date, three studies have attempted to characterize the relationship between acarological risk and human incidence, but these have been only examined a single state. We recently completed a 3-year field-based acarological risk map for Lyme infection in the form of an 8x8km surface for the complete distribution of I. scapularis, east of the 100th meridian. Here, we assess the predictive value of model-derived acarological risk on Lyme disease incidence in 25 US states. We used a generalized linear model with poisson errors and county population size as an offset. Average acarological risk per county predicted Lyme disease incidence in endemic areas (R2 0.39). Including state as a fixed effect improved the model fit (R2 0.61) and state-by-state significant differences in the relationship between acarological risk and Lyme disease incidence were identified. Running the model for individual states, significant positive log-linear relationships were found in 10 endemic states while a significant negative relationship was found in one state. The relationship of acarological risk and incidence could not be described with a log-linear model in 6 endemic states. Acarological risk was also a significant predictor of incidence in all endemic and non-endemic states combined. Further analyses of the relationship between acarological risk and incidence may provide insights into additional key factors driving the current Lyme disease epidemic, such as landscape structure affecting human exposure to ticks and genetic variation in *B. burgdorferi* linked to differential human pathogenicity.

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RICKETTSIA AESCHLIMANNII INFECTION AMONG PATIENTS WITH ACUTE FEBRILE ILLNESS IN KENYA

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Rickettsial diseases are distributed worldwide and are caused by infections with intracellular obligate bacteria that are transmitted to humans by arthropod vectors. Increasing reports of rickettsioses among the local population of and international travelers to Kenya prompted this study. Blood samples were collected from patients presenting with acute febrile illness at hospitals that serve the nomadic population of the Rift Valley (Baringo District Hospital) and Northeastern Province (Iftin and Garissa Police-line sub-District Hospitals), Kenya. Serum samples (n=220) were first screened for antibodies against spotted fever and typhus group specific rickettsiae by ELISA to determine exposure. DNA was extracted from whole blood (EDTA) and assessed by qPCR for presence of the 17 KDa genus-specific protein gene. All qPCR positive DNA samples were amplified and sequenced with primers sets that targeted rickettsial outer membrane proteins A and B genes (ompA and ompB) and the citrate synthase encoding gene (gltA). Species identification was further confirmed by a species-specific qPCR assay. Using ELISA, 29.5% (65/220) and 0.9% (2/220) of the patients had at least 1:400 antibody titer to IgG against spotted fever and typhus group rickettsiae, respectively, while 13 of 220 (5.9%) had active rickettsial infections as determined by a positive genus-specific qPCR assay. These infections were confirmed to have been caused by *Rickettsia aeschlimannii* by molecular sequencing of ompA, ompB and gltA genes and further verified by a *R. aeschlimannii*-specific qPCR assay.

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DETECTION OF A UNIQUE *RICKETTSIA* IN FLEAS FROM ASEMBO, KENYA

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The flea-borne rickettsioses, murine typhus (*Rickettsia typhi*) and flea-borne spotted fever (*R. felis*), produce febrile diseases among humans worldwide. *R. typhi* has been known to be endemic to Kenya since the 1960's, but *R. felis* was only recently documented in Kenya. To assess the risk of flea-borne disease to humans in western Kenya, fleas collected from domestic and peri-domestic animals, and from human dwellings, were tested for rickettsiae. The study took place in Asembo in Rarieda District of western Kenya, a rural site bordering Lake Victoria. Fleas were collected from dogs, cats, and rodents, and from human dwellings using light traps. DNA was extracted from fleas and assessed for the presence of rickettsiae by two genus-specific quantitative real-time PCR (qPCR) assays based upon the 17 kDa antigen and citrate synthase genes. In addition, the species-specific qPCR assays for *R. typhi* and *R. felis* were used. Multilocus sequence typing (MLST) targeting the 16S rRNA, 17 kDa antigen, citrate synthase and outer membrane protein B genes was performed on selected positive samples representing different locations and host species. Of 134 pools of fleas tested, 80 (59.7%) were positive for rickettsiae. All 80 samples were also positive by the R. felis-specific qPCR assay. MLST determined that, of 12 positive samples, one was *R. felis* and the other 11 were a unique Rickettsia similar to Rickettsia RF2151, a previously-described rickettsial agent of fleas and mites. Characterization of this new agent by MLST shows that it is closely related to R. felis but genetically dissimilar enough to be considered a separate species. Fleas collected from Asembo, Kenya contain the rickettsial pathogen R. felis, the causative agents of flea-borne spotted fever. In addition, a new rickettsia, similar to one described previously from Thailand, South Carolina, Egypt and Hungary (i.e. Rickettsia RF2151), was identified and characterized.

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ETIOLOGY AND SEASONALITY OF VIRAL RESPIRATORY INFECTIONS IN RURAL HONDURAN CHILDREN

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Limited data are available in Honduras describing the etiology and seasonality of childhood acute respiratory infections (ARIs), and better data may lead to improved therapeutic and preventative strategies. We conducted a prospective sentinel clinic surveillance study to determine the viral etiology of ARIs in rural Honduran children less than 5 years of age to characterize the spectrum and seasonality of viruses associated with acute respiratory infections. Nasopharyngeal samples were obtained via flocked swab and shipped to the U.S. in both universal transport medium (UTM) on dry ice and a nucleic acid stabilizing buffer at room temperature. Samples were tested for 14 respiratory viruses using a commerically available PCR respiratory viral panel. 267 samples were collected from February 2010 - March 2011; 13.9% were positive for influenza, 7.9% for metapneumovirus, 7.5% for respiratory syncytial virus (RSV), 7.1% for parainfluenza and 2.2% for adenovirus. At least one virus was identified

in 194 (72.7%) cases, of which 16 (6.0%) were co-infections. Influenza rose from 1.8% of isolates in February through June to 25.7% of isolates in July through October. No cases of influenza were identified from November 2010 through February 2011. Influenza was present for 5 out of 12 months, and influenza correlated with monthly rainfall in millimeters ($R^2 = 0.2857$). Including all tested respiratory viruses except enterovirus/rhinovirus, the presence of a respiratory virus positively correlated with average monthly precipitation ($R^2 = 0.2863$). Influenza results for UTM on dry ice and nucleic acid stabilizing buffer at room temperature correlated well ($K = 0.767$, $p<0.0001$). In conclusion, these unique results suggest that the spectrum of viruses in rural Honduran children is similar to those found in the U.S., though the seasonality is tropical. This region of rural Honduras demonstrated one large peak in influenza positivity prior to the peak in the U.S., and influenza and respiratory viruses in general correlated with average monthly rainfall. Nucleic acid stabilizing buffer at room temperature is an effective shipping method for subsequent isolation of influenza as compared to UTM. Further research is needed to determine the best methods of prevention and treatment of these viral respiratory infections.

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INCIDENCE OF HOSPITAL-ACQUIRED INFLUENZA IN BANGLADESHI TERTIARY CARE HOSPITALS, 2008-2011

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Infection control is a challenge in all healthcare settings, particularly in low-income countries where infection control resources are scarce. This study aimed to determine the incidence of hospital-acquired influenza in three tertiary care hospitals in Bangladesh. During May 2008 - March 2011, surveillance physicians followed patients admitted >72 hours in adult and pediatric medicine wards and collected nasal and throat swabs from patients with new onset of fever, cough, runny nose, or difficult breathing at three hospitals. Surveillance physicians followed patients until death or discharge and counted the number of patients hospitalized and patient-days at risk. Swabs were tested for influenza A and B viruses using real-time RT-PCR. We calculated the frequency and incidence per patient-day of hospital acquired influenza infection. Approximately 22,000 patients were hospitalized >72 hours representing ~150,000 patient-days at risk. Incidence of hospital-acquired respiratory infections was 3.8 per 1000 patient-days at risk. We collected specimens from 563 patients; 55 (10%) were positive for influenza virus and 64% (35/55) were influenza A. Mean days from admission to onset of influenza illness was 7.6 days (median 7). The most commonly identified influenza A subtype was H3 (16/35), followed by pH1N1 (10/35), and seasonal H1 (9/35). Overall, the incidence of hospital acquired influenza was 3.7 per 10,000 patient-days at risk. Highest rates occurred in the pediatric wards (7/10,000) compared to adult male (4/10,000) and adult female (3/10,000) wards. We identified 8 patient deaths associated with new onset of respiratory symptoms; 2 were associated with hospital acquired influenza. One male aged 60 years admitted for COPD died with influenza B infection with onset 8 days post-admission and one women aged 65 years admitted with renal failure died with influenza A/H3 infection with onset 5 days post-admission. Approximately 1 per every 40 patients admitted >72 hours in these hospitals developed new onset of respiratory symptoms and our study found that 10% of those infections were caused by influenza virus. Given the serious health outcomes that can occur during influenza infections and lack of vaccine in this setting, better evidence about the effectiveness of non-pharmaceutical interventions to prevent hospital acquired influenza is needed.

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HIGH INCIDENCE OF INFLUENZA AND DEFINED SEASONALITY IN A COHORT STUDY OF NICARAGUAN CHILDREN

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Historically, influenza has not been considered a major health problem in the tropics; however, recent data indicate that there is a high burden of influenza in tropical developing countries. Additionally, other epidemiologic features, such as seasonality of influenza in these countries, is poorly understood. To examine the incidence, epidemiologic characteristics, and transmission of influenza virus infection in children in Managua, Nicaragua, we established a prospective cohort study of influenza, conducted between June 2007 and December 2011. The study population consisted of ~3,800 children aged 2-14 years. Participants were encouraged to come to the study health center at the first sign of illness, and 94% presented during the first 72 hours since onset of symptoms. All medical care was provided free-of-charge, and data was systematically recorded on all medical visits. Samples from 25% of all participants presenting with influenza-like illness (ILI) -- fever or reported fever with cough or sore throat or rhinorrhea -- were tested for influenza viruses by RT-PCR. Viral isolation was performed on all RT-PCR-positive samples. Weekly influenza incidence in the cohort was estimated by multiplying the percentage of samples positive for influenza in the calendar week by the total number of children who presented with ILI, divided by the person-time for that week. Yearly incidence varied from 15.5 to 27.5 cases per 100 person-years, with an estimated incidence of 20.0 cases per 100 person-years in the 3 years of the study. A defined seasonality was observed in all years with peak seasonal influenza activity occurring in June-July, and low level transmission occurring sporadically at other times during the year. Influenza B activity peaked later than seasonal influenza A, which consisted of H3N2 in 2007, H1N1 in 2008, and H3N2 in 2010. The first case of pandemic influenza A H1N1 2009 in Nicaragua was detected in the cohort on June 1, 2010; however, peak levels of pandemic influenza did not occur until August, with H1N1 2009 cases detected until October 2010. These data demonstrate that Nicaraguan children experience a substantial burden of influenza and that a defined seasonality of influenza occurs annually in Nicaragua. Additionally, transmission dynamics of pandemic influenza differed from those observed for seasonal influenza. This study provides critical data on the epidemiology and transmission of influenza in the Americas.

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VIRAL ETIOLOGIES OF INFLUENZA-LIKE-ILLNESSES IN KENYA; JANUARY 2007 TO DECEMBER 2010

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In Kenya, little is known about the etiology and prevalence of viruses causing influenza-like illnesses (ILIs). We conducted a four year study of the viral etiologies of influenza-like illnesses in Kenya among persons from 2 months onwards. Nasopharyngeal swabs were collected from patients who presented with Influenza like illnesses in our 8 sentinel surveillance hospitals. ILI was defined as fever >38ºC, cough or sore throat, onset of ILI within the previous 72 hrs. Clinical and epidemiologic Information

of each patient was gathered using a questionnaire. We screened the specimens using real time RT-PCR, IFA, HA/HAI. Between 1st January 2007 and 31 December 2010, 12, 938 patients were recruited. A total of 2,999 viruses were identified. Of these, 1,345 (44.8%) were influenza consisting of 388 (12.9%) influenza A/H3N2, 161 (5.4%) influenza A/H1N1, 258 (8.6%) influenza A pH1N1 and 538 (17.9%) influenza B. 635 (21.2%) were human parainfluenza viruses, 308(10.3%) were RSV, 444 (14.8%) were human Adenoviruses and 267 (8.9%) were Enteroviruses. PIVs were the most prevalent single virus at 5 of the 8 sites while influenza B was the most common at the other 3 sites. Overall, influenzaviruses were the most common group causing ILI at all the sites. Influenza B and influenza A/H3N2 showed distinct seasonality and were prevalent betweeen March and August of each of the years under study. All the other viruses were identified throughout the year with no distinct seasonality. These results provide evidence of the importance and the diversity of viruses as causative agents of acute respiratory infections in Kenya. The findings generally show that influenza viruses are the most common cause of ILI in the country with a prevalence generally higher than global findings. In conclusion, we found that respiratory viruses play an important role in ILI's in Kenya. The data provide a better understanding for the first time in the country, of the viral etiolologies of outpatients with ILI and their seasonality.

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INDOOR EXPOSURES TO RESPIRABLE PARTICULATE MATTER AND AGE AT FIRST PNEUMONIA EPISODE IN A LOW-INCOME, URBAN COMMUNITY IN BANGLADESH

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Acute lower respiratory infection (ALRI), including pneumonia, is the leading cause of death in children <5 years of age in Bangladesh. Infants have the highest risk of pneumonia mortality but the potentially modifiable risk factors that predispose them to this risk are not well described. This study aimed to estimate the effect of increased exposure to indoor respirable particulate matter on the age that a child develops their first pneumonia episode in a low-income urban community in Bangladesh. A cohort of 235 children were enrolled at birth during December 2007 - April 2009 and followed at home twice every week through 2 years of age. Children with either one major or two minor signs of illness were referred to a free study clinic. We defined pneumonia as cough or difficulty breathing with tachypnea. Particulate matter approximately 2.5 micrograms in diameter ($PM_{2.5}$) was measured above the child's bed using a portable monitor for one 24-hour period each month during May 2009 - April 2010 to characterize the air quality in the home. We described the age distribution of first pneumonia episodes and fit a parametric survival model to estimate the change in age at first pneumonia episode associated with increased exposure to indoor $PM_{2.5}$, controlling for possible confounding of household wealth, mother's education, crowding, and low birth weight. One hundred and forty-six of 235 (62%) children experienced at least one episode of pneumonia during follow-up; 25% of children experienced their first episode by 3 months of age and 50% by 10 months. Mean annual $PM_{2.5}$ concentrations were 200 μg/m^3 in children's sleeping spaces. For the 146 children who experienced their first episode of pneumonia during the study, each additional hour that indoor $PM_{2.5}$ exceeded 100 μg/m^3 was associated with a 6% decrease in age at first pneumonia episode after controlling for confounders (95% CI 12% - 0%, p=0.07). Numerous epidemiologic studies have found an increase in pneumonia risk associated with increased exposure to indoor air pollution. Our findings suggest that increased exposure to $PM_{2.5}$ may also be associated with younger age at first pneumonia episode, placing a particularly vulnerable age group at increased risk of severe disease or death.

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WORLDWIDE SPREAD OF 2009 INFLUENZA A (H1N1) DURING 2009-2010 AND THE EFFECT OF SEASONALITY ON TRANSMISSION

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Yearly influenza transmission patterns vary throughout the world with time of year and region. Understanding how 2009 influenza A (H1N1) (H1N1pdm) activity varied in relation to geographic regions and time of year, might help us understand the behavior of future pandemic viruses. We obtained laboratory influenza strain data by country from April, 2009-August, 2010 compiled by the World Health Organization. We selected countries with data for ≥ 70% of weeks during that period and that reported >120 positive H1N1pdm samples. We assessed influenza activity by calculating the weekly proportions of samples positive for influenza A subtypes out of all influenza-positive samples, identified the peak of H1N1pdm proportion positivity and number of weeks from initial H1N1pdm detection until the peak week. We compared these parameters between countries in different climatic regions. Timing of the traditional influenza season was known for 39 countries from a prior study. We compared the parameters of H1N1pdm activity between countries where first H1N1pdm detection coincided with the start of the country's traditional influenza season versus countries where first detection occurred at a different time of the year. We quantified weekly percents of H1N1pdm out of influenza A-positive specimens, and correlated country medians for the pandemic period with their central latitude. We analyzed data from 80 countries and administrative regions. The median peak H1N1pdm proportion positivity was significantly different between temperate (0.17), subtropical (0.1) and tropical (0.11) regions (p=0.0002). For countries where H1N1pdm was first identified during the start of their traditional influenza season, it took a median of 8 weeks from first H1N1pdm detection until the week of peak proportion positivity, versus 22 weeks for countries where first detection occurred at any other time of the year (p=0.0007). Our data suggests a positive correlation between country medians of weekly H1N1pdm percents and country central latitudes, though this was not statistically significant (Pearsons correlation=0.35, p=0.08). Our findings suggest that temperate countries may have higher peak pandemic activity than other countries. Countries that first identify pandemic strains at the start of their influenza season may have fewer weeks to prepare before facing peak pandemic activity. Latitude may have an effect on pandemic strain predominance.

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DETERMINANTS OF ANTIBIOTICS PRESCRIPTION IN SCHOOLCHILDREN AT ALLADA, SOUTH BENIN

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Implementation of malaria rapid diagnostic tests (mRDT) has been repeatedly associated with an increase of antibiotics prescriptions. We aimed to study determinants of antibiotics prescriptions to schoolchildren by nurses in South Benin. Data were collected in the setting of a prospective study on treatment of malaria restricted to parasitologically-

confirmed cases. Children were included from February until June 2008. For each patient, sociodemographic characteristics, complaints, final diagnosis established by nurses and therapeutic prescriptions were collected. Assessment of malaria was performed with mRDT. Data were entered and validated with Epidata® software, and analyzed with STATA 10® software. One thousand six hundred thirty children were included. Fever was the first reason for consultation (57 %), followed by the digestive symptoms (27 %), respiratory symptoms (24 %) and skin lesions (17 %). A malaria diagnosis was made in 61 % of the children attending for fever. Forty percent of children were prescribed an antibiotic (21 % of children with a malaria diagnosis and 57 % of children with a non-malarial-fever). We found a very significant association between an antibiotic prescription and a respiratory infection diagnosis (OR [IC 95 %]: 41.09 [24.34-69.33]), and to a lesser extent between an antibiotic prescription and a cutaneous infection diagnosis (OR [IC 95 %]: 5.78 [4.20-7.97]). The rational use of antibiotics in malaria endemic areas has become an even more critical issue, since the implementation of mRDT is boosting antibiotics prescriptions. Analyzing determinants of antibiotics prescription is a first step on the way to rationalize antibiotics prescriptions. We found that, by far, the diagnosis of respiratory infection is the main factor associated with an antibiotic prescription. Further clinical research studies are needed in order to develop algorithms aimed at selecting among children who complain with respiratory symptoms, those who should be prescribed antibiotics.

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ALTERATIONS IN THE *AEDES AEGYPTI* TRANSCRIPTOME DURING INFECTION WITH WEST NILE, DENGUE AND YELLOW FEVER VIRUSES

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West Nile (WNV), dengue (DENV) and yellow fever (YFV) viruses are globally important, re-emerging mosquito-borne flaviviruses that cause widespread human disease and mortality. Flaviviral genomes encode relatively few proteins, and hence likely manipulate host gene expression to facilitate infection. The influence of infection on mosquito gene expression - both common and unique to individual flaviviruses - is poorly understood. Here we present a comprehensive microarray analysis of the *Aedes aegypti* transcriptome on days 1, 2 and 7 (D1, D2, D7) post infection (p.i.) with DENV, WNV or YFV. 203 mosquito genes were ≥5-fold differentially up-regulated (DUR) and 202 genes were ≥10-fold differentially down-regulated (DDR) during infection. Most are newly identified as flaviviral mosquito host factors, however several of the genes were previously identified as host factors for WNV and/or DENV in human cells, mosquito cells and live mosquitoes, including serine protease inhibitors and chitin-binding proteins. We also demonstrate that virally-regulated gene expression is tissue-specific. Bioinformatics analysis revealed changes in expression of genes from diverse cellular processes, including ion binding, transport, metabolic processes and peptidase activity. Comparative analysis revealed that the expression profile of 20 DUR genes and 15 DDR genes is highly similar between the three flaviviruses on D1 of infection, indicating a potentially conserved transcriptomic signature of flaviviral infection. The overexpression of several genes that were down-regulated during flavivirus infection decreased WNV infection both in mosquito cells and live *Ae. aegypti* mosquitoes. This work provides an extensive list of targets for controlling flaviviral infection in mosquitoes that may also be used to develop broad preventative and therapeutic measures for multiple flaviviruses.

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ROLES OF VENOM PROTEINS IN THE TRANSMISSION OF FLAVIVIRUSES

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Similar to other vector-borne diseases, flaviviruses are transmitted to their host covered in vector saliva. Vector saliva has been shown to enhance the transmissibility of many vector-borne pathogens. To reveal genes that are modulated during infection with multiple flaviviruses, we performed microarray analyses on West Nile, dengue, and yellow fever virus-infected and uninfected, whole *Aedes aegypti* mosquitoes. We discovered 20 genes that were upregulated during infection with all three viruses that had a predicted extracellular localization. One of these genes had a paralogue on the most recent sialotranscriptome. BLAST analysis identifed 20 additional paralogues in the *Ae. aegypti* genome that we describe as trypsin inhibitor-like domain-containing cysteine-rich venom proteins (TIL-CRVPs). Each paralogue has a signal peptide and is enriched in salivary gland tissue compared to midgut tissue. RT-qPCR analysis of each paralogue suggests that TIL-CRVPs are differentially modulated by West Nile virus and dengue virus infection in salivary glands. Modulation of expression also appears to be specific to the salivary glands. Further research into the roles of TIL-CRVPs in infectivity and transmission of multiple flaviviruses will be discussed.

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CHARACTERIZATION OF PERSISTENT WEST NILE VIRUS INFECTION IN HOUSE SPARROWS (*PASSER DOMESTICUS*)

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West Nile virus (WNV) is now endemic throughout North America, a feat dependent on the ability to persist through temperate winters that drive mosquito vectors into inactivity and halt the transmission cycle. We hypothesize that persistent WNV infections in selected avian hosts may provide an overwintering opportunity for the virus. Evidence that wild birds develop persistent WNV infections has been mounting, but characterization of these persistent infections has been lacking. To further investigate WNV persistence, House Sparrows were experimentally infected with WNV and held in groups of 8-10 for 3, 5, 7, 9, 12, 15, or 18 weeks post infection. Blood was collected every two weeks and sera tested for antibodies against WNV and RNA specific for WNV. At the end of each holding period, groups were necropsied and assessed for the presence of persistent WNV. Infectious virus was detected using a Vero cell cocultivation technique and RNA was detected using qRT-PCR. WNV RNA was present in the sera of some birds for up to 7 weeks post infection and in the tissues of birds at necropsy at all time points (aside from 15 weeks). Infectious virus was isolated from the spleen of birds held 3, 5, 7 and 12 weeks post infection. Our findings confirmed that some House Sparrows and perhaps other species can develop persistent WNV infections and that these infections potentially may serve as an overwintering mechanism for the virus. Planned research will determine whether these persistent infections recrudesce and are able to infect mosquitoes thereby restarting the vernal WNV transmission cycle.

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SMALL RNA SEQUENCING OF THE ANTI-WNV RESPONSE IN FREE-RANGING *CULEX QUINQUEFASCIATUS* MOSQUITOES: EVIDENCE FOR STEREOTYPICAL TARGETING OF THE VIRAL GENOME AND COMPARISON TO COLONIZED MOSQUITOES

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Small RNA regulatory pathways are an integral component of endogenous post-transcriptional regulation of gene expression as well as innate immunity. In particular, the RNA interference (RNAi) pathway functions as the innate response to arbovirus infection in vector mosquitoes and influences virus diversification and mosquito vector competence. However, most studies of RNAi in mosquitoes have focused on relatively inbred, colonized mosquitoes. We tested the hypothesis that free-ranging *Culex quinquefasciatus* mosquitoes would mount an RNAi response in response to West Nile virus (WNV) infection by collecting egg rafts from two sampling sites, rearing the resulting mosquitoes under standard laboratory conditions, exposing them to WNV in an artificial bloodmeal, and holding the mosquitoes for 14 days extrinsic incubation. We then used the Illumina® deep-sequencing platform to profile the small RNA (sRNA) populations from pools of five infected midguts for each sampling site, and compared sRNA profiles from free-ranging mosquitoes to colonized, WNV infected mosquitoes. We found that, similar to colonized *Cx. quinquefasciatus*, 21 nucleotide RNAs were the most common sRNA targeting the WNV genome; however, the overall distribution of sRNA reads between 19-30 nucleotides in length varied, with differences primarily seen in the 21 nucleotide and 24-30 nucleotide species between the three groups. We also found that the intensity of viRNA targeting of the WNV genome was correlated across all three groups of mosquitoes, but that the correlation was strongest between the two free-ranging groups. These data help illustrate that mosquitoes respond to WNV infection through a stereotypical, RNAi-based response, and that certain regions of the virus genome are prone to much heavier targeting by the RNAi pathway than others. However, important variation in viRNA targeting exists, which may influence virus diversification and/or vector competence.

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THE APPLICATION OF CELL-SPECIFIC RNA SILENCING AND CODON DEOPTIMIZATION AS TOOLS FOR THE RATIONAL DESIGN OF LIVE VIRUS VACCINES

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Conventional strategies for the development of live-attenuated viral vaccines, such as culture adaptation and virus recombination often yield vaccines with variable degrees of residual pathogenicity, immunogenicity and phenotypic stability. Consequently, it is becoming increasingly necessary to define the specific determinants responsible for virus attenuation in newly-developed vaccines. This requirement and the inherent uncertainty in the application of these strategies can cause significant delays in vaccine production. To address these problems we have developed a vaccine platform that permits the rapid and intuitive design of viral vaccines. Attenuation of viruses is achieved by the introduction of large complements of rare codons into the viral ORF that restrict protein translation by sensitizing the virus to limiting pools of cellular tRNAs. To further enhance the safety of these vaccines we introduced target sequences for cell-specific miRNAs into the viral 3′ UTR. This approach specifically abrogates infection of cells that are associated with disease pathology. In the current study we evaluated the applicability of this approach for the development of LAV vaccines for West Nile virus (WNV). As many as 293 rare codons were introduced into WNV resulting in greater than a 1,000-fold attenuation in 3 week-old CD-1 mice. A single vaccination with WNVΔRC293 engendered complete protection against lethal challenge with wild-type WNV. Through the introduction of cell-specific miRNAs into WNV we were able to demonstrate that infection of neurons is critical to WNV neuroinvasion. Selective blockade of neuronal infection by WNV completely abrogated disease in mice while permitting induction of a potent protective humoral response. This study highlights the potential of these strategies for the rational design of LAV vaccines.

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USE OF MOSQUITO-SPECIFIC MICRORNAS FOR RESTRICTING VECTOR INFECTIVITY OF WEST NILE VIRUS: IMPLICATIONS FOR LIVE-ATTENUATED VACCINE DEVELOPMENT

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MicroRNAs (miRNAs) serve a key role for post-transcriptional gene regulation in eukaryotic hosts. Since genomes of positive-strand RNA viruses are equivalent to host mRNAs in structure and are present within the cytoplasm of infected cells, the feasibility of harnessing this regulatory pathway for restricting viral replication in different host cells by incorporating multiple copies of host tissue-specific miRNA target sequences into the 3′UTR of a West Nile virus (WNV) infectious cDNA clone was explored. Mutant WNVs were generated from these constructs that incorporated three copies of neuron, myeloid, muscle and mosquito-specific miRNA target sequences. Growth profiles of these recombinant viruses were compared to wild type virus lacking any miRNA target sequence insertion both *in vitro* and in mosquitoes. Viral titers observed following infection with the parental WNV and the recombinant miRNA target sequence-inclusive viruses were indistinguishable in Vero cells not expressing any of the miRNAs for which target sequences were engineered; however, no replication of the WNV containing the mosquito-specific miRNA target sequence was observed in *Aedes albopictus* or *Culex tarsalis* mosquito cells, while the parental WNV grew to titers over 7 $\log_{10}$ PFU/mL. Infection of *Cx. quinquefasciatus* mosquitoes was not impaired for viruses containing the neuron, myeloid or muscle-specific miRNA target sequences compared to the wild type WNV. In contrast, a75% reduction in the infection rate was observed for the WNV containing the mosquito-specific miRNA target sequences. Furthermore, none of the mosquitoes infected with this construct exhibited a disseminated infection or was capable of transmitting virus compared to dissemination and transmission rates of at least 92% and 87%, respectively, for the parental WNV and alternative miRNA target sequence mutants. *In vitro* and *in vivo* results presented herein indicate the utility and specificity of the miRNA mediated gene silencing approach for blocking mosquito infectivity of live attenuated vaccine candidates.

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AUTOPHAGY FUNCTIONS IN A PRO-VIRAL CAPACITY DURING WEST NILE VIRUS INFECTION OF MOSQUITO CELLS

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Autophagy is an evolutionarily conserved process that mediates the transfer of cytoplasmic materials to lysosomes for degradation. This pathway serves an important role in maintaining cellular homeostasis and cell survival. In addition, autophagy functions as an innate immune defense against intracellular pathogens, such as *Mycobacterium tuberculosis* and *Listeria monocytogenes.* Interestingly, the role of autophagy during viral infections has been implicated as having both

pro- and antiviral activity. Surprisingly, the role of autophagy during arbovirus infection of vector mosquitoes is unknown. Therefore, we investigated the role of autophagy during West Nile virus (WNV; *Flaviviridae*, *Flavivirus*) infection of C6/36 *Aedes albopictus* cells and hypothesized that autophagy functions in a pro-viral capacity during WNV infection of mosquito cells. C6/36 cells were either untreated or treated with an autophagy inducer (pp242) or an autophagy inhibitor (3-methyladenine (3-MA)) and infected with WNV. Extracellular WNV titers were not significantly affected by pp242, however, in the presence of 3-MA, WNV titers were reduced nearly two logs. This data suggests that autophagy is required for optimal WNV replication within mosquito cells. Furthermore, we found that WNV induces autophagy in mosquito cells as the ratio of the LC3-II to LC3-I was significantly increased as determined by immunoblot. In addition, using electron and confocal microscopy analysis, we found that WNV infection not only increased the number of autophagosome positive cells, but also increased the average number of autophagosomes per cell. Our findings provide evidence, for the first time, that WNV utilizes the autophagy pathway for efficient replication during infection of mosquito cells and lay a foundation for future work that may be translated to other virus/ vector pairings.

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SUBARACHNOID NEUROCYSTICERCOSIS: EXPERIENCE FROM AN INNER CITY HOSPITAL IN THE BRONX

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Subarachnoid cysts in Neurocysticercosis (NCC) can grow as membranous or cystic masses and elicit an exuberant host response leading to complications such as hydrocephalus or stroke and requiring longer or repeated treatments compared to parenchymal disease. A retrospective review of patients with subarachnoid disease seen in our tropical medicine clinic since 1997; treatments and outcomes are described here.20 patients (mean age 41±13 years ; 17 males) were followed. All but one were from endemic regions (Mexico, South America, Eastern Europe, SE Asia). Mean time from immigration was 12±9.3 years. Presenting symptoms were headache (90%), ataxia (70%), focal neurologic complaints (70%), and altered mental status (45%). Complications included hydrocephalus (80%), vascular events (55%), and seizures (35%); 11 patients required ICU stay. VP shunts were placed in 13 patients;3 were complicated by infection. All patients had involvement of the basilar subarachnoid space. Spinal involvement was found in 6/15 patients imaged. MRA was performed in 12 patients and revealed abnormal findings in 3. All patients received steroids and Albendazole.Methotraxate was used in 10 patients as a steroid-sparing agent. 14 patients completed a 1 year follow-up :4 had resolved on MRI (average - 10 months therapy) and 10 had continued enhancing cysts; 3 patients currently have unresolved disease at 6 months of therapy; 3 were lost to follow up. Overall, despite clinical improvement and continued treatment, active MRI lesions were found on 85% at 3 months, 76% at 6 months, and 75% at 9 months. Mean treatment duration was 35±24 weeks for steroids, 48±32 weeks for Albendazole, and 28±27 weeks for Methotrexate. Patients treated with repeated courses required longer therapy compared to those on a single extended course (p=0.01). In conclusion, patients with subarachnoid NCC experience serious complications and require prolonged treatment when MRI is used to guide duration of therapy. Further studies are required to establish the optimal treatment for this subset of patients.

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PERI-CYSTIC INFLAMMATORY RESPONSES ARE ASSOCIATED WITH LOSS OF VASCULAR INTEGRITY IN PORCINE NEUROCYSTICERCOSIS

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Humans and pigs are the two natural hosts for the cestode *Taenia solium*, the etiological agent for neurocysticercosis (NCC). Infection in pigs results in high cyst burdens in the brain, but the nature and severity of inflammation in the brain to degenerating cysts and paucity of inflammation to viable cysts are poorly understood. To investigate the relationship between vascular leakage around cysts and the host response to the cysts, pigs were injected with Evans Blue (EB), which caused blue staining of some pericyst tissues indicating blood brain barrier disruption. Pericyst brain tissues were fixed for histopathological analysis and RNA subjected to real time PCR to quantify the expression of genes for proinflammatory (IL-2, CD80, IFN-γ, TNF-α, IL-2Ra, etc), regulatory (IL-2Ra, FoxP3, IL-10, CTLA4) and granuloma-associated (MMP1, MMP9, TIMP1, TIMP2 and SPP1) cellular markers; expression around blue-stained cysts were compared to corresponding expression levels in brain tissues around non-blue stained cysts and from uninfected tissues. Preliminary results from a total of 21 pericystic samples, 4 samples from uninfected pigs and 2 samples from unaffected brain tissue from infected pigs indicated that proinflammatory responses (including IL-2, IFN-γ, CD80, and IL-2Ra), but not TNF-α, were unregulated in tissue around cysts compared to uninfected tissues. This response was accompanied by increased expression of regulatory genes (IL-10, CTLA4), but not in FoxP3 expression (indicative of T regulatory cell populations). The differences in gene expressions were most apparent in cysts that demonstrated vascular leakage, as determined by EB staining. Analyses of additional pericystic samples are under way to confirm that differences observed are statistically significant and the role of regulatory responses in protecting against inflammatory pathology. These findings indicate that inflammatory responses are increased around cysts that show loss of vascular integrity, and provide a means to elucidate the mechanisms of inflammation.

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SINGLE BRAIN LESION AND ANTIBODIES TO CYSTICERCOSIS ON EITB

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A single brain enhancing lesion is a clinical entity commonly associated with seizures in developing countries, and particularly in the Indian subcontinent. Cysticercosis is the major etiological contributor. The serological test of choice for cysticercosis is an immunoblot (EITB) using lentil-lectin purified glycoprotein antigens. This assay, detecting one to

seven specific antibody bands, is 98% sensitive and 100% specific in cases with multiple lesions, although sensitivity drops to 70% or less in cases with a single enhancing lesion. Systematic series of cases of single brain enhancing lesions outside of India are scarce. From 1997 to 2008, 1940 patients with suspected neurocysticercosis (NCC) attended the Cysticercosis Unit of the Instituto Nacional de Ciencias Neurologicas in Lima, and had at least one brain image compatible with NCC. A single lesion was found in 514 (26%). Of these, 173 (9%) had a single viable cyst (with defined liquid content, with or without surrounding inflammation); 132 (7%) had a single enhancing lesion, and another 209 (11%) had a brain calcification. Seropositivity to cysticercosis on EITB was assessed in 359 cases who also had an archive serum sample +/- 30 days from the image. Seropositivity on EITB was 76.7% for cases with a viable cyst (n=142), 63% in cases with a single enhancing lesion (n=102), and 56% in cases with a brain calcification (n=115). Older patients (>40 for a enhancing lesion, and >20 for a viable cyst) were more frequently seropositive. We had access to 144 actual films from the 244 cases with active lesions and corresponding serum samples. The numbers of reactive bands on EITB were strongly associated with the volume of viable cysts ($p<0.005$) but not for single enhancing lesions. Positive serology is more frequent in cases with a single viable brain cyst than in those with an enhancing lesion, but other variables including the age of the patient and the volume of the lesion influence the antibody responses.

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DEVELOPMENT OF A MULTIPLEXED BEAD BASED IMMUNOASSAY FOR DIAGNOSIS OF NEUROCYSTICERCOSIS

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Establishing a diagnosis of neurocysticercosis relies on imaging and laboratory methods that detect *Taenia solium* specific antibodies using the CDC developed enzyme -linked immuno electro transfer blot (EITB). Recently, we have systematically generated recombinant proteins or synthetic peptides that represent the diagnostic proteins recognized in the EITB as well as taeniasis specific protein antigens. In this study, we developed a Luminex based multiplex assay to simultaneously detect antibody responses to 6 cysticercosis (rGP50 and rT24H, sTsRS1, sTS18var1, sTSRS2var1, and sTS14) and 2 taeniasis (rES33 and rES38) specific proteins. We coupled MagPlex magnetic particles, using the EDC-Sulfo-NHS coupling, to the recombinant protein or synthetic peptide antigens. We tested 245 presumed negative sera collected from persons with no reported travel history outside of the United States, 128 sera from patients with neurocysticercosis with 2 or more viable cysts, and 185 sera from persons with confirmed *T. solium* taeniasis. When responses to the individual antigens was examined, the sensitivity of sTs18var1 was 99% for immunodiagnosis of neurocysticercosis cases with 2 or more cysts, with a specificity of 96%; rGP50 and rT24H were 94% and 91% sensitive and 96% and 94% specific, respectively. When responses to a combination of antigens were used, sTs18var1, rGP50, and rT24H, the assay sensitivity and specificity were 99% and 91%, respectively. For immunodiagnosis of neurocysticercosis cases with a single viable cyst, antibodies to sTs18var1 were detected in 69% of the specimens. Using a combination of antigens, sTs18var1, rGP50, and rT24H, the sensitivity for detection of cases with a single cyst improved to 92%. Immunodetection of taeniasis cases was unsuccessful using this methodology; rES33 performance was poor with a sensitivity of 61% and a specificity of 79% and rES38 produced no signal at all. In conclusion, the performance of the Luminex bead based assay is excellent and is comparable to other assays using native or recombinant proteins. The assay show s a marked improvement over all other described methods for detection of neurocysticercosis cases with single lesions.

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PERFORMANCE OF SEROLOGICAL AND PCR EXAMINATIONS OF THE SPINAL CORD FLUID FOR THE DIAGNOSTIC OF NEUROCYSTICERCOSIS

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Neurocysticercosis (NCC) is the most important cause of seizure in tropical countries. In Madagascar seroprevalence of cysticercosis (in blood) can reach 20% in population of the highlands. However biological methods used in blood can give high level of false positivity due to extra-cerebral localisation of the cysts, and can be in same time poorly sensitive when a single cyst is located in brain. Scanners are also poorly available in tropical countries leading treatment of the patients with anti-helminths on behalf of ELISA anti-Ts results. To improve the diagnostic of NCC for patients suffering from recent seizure or cephalae in Madagascar, we developed real time PCR method detecting the T.solium COX1 gene and we used it in parallel with a western blot analysis of the spinal cord fluid (SCF). The performance of these methods was compared with the scanographic examination of the patients. Sensitivity of the Q-PCR method for the detection of *Taenia solium* DNA was established by its own using serial dilution of DNA extracted from cyst, re-diluted in SCF before re-extraction. 0.2 pg/ml of *T. solium* DNA can be detected with less than 40 PCR cycles. Specificity was check using SCF from patients suffering from other pathologies and with DNA from other parasites. WB was conducted according to Tsang et al (1989). To date 105 patients attending the clinic of neurology for recent seizure and/or recent and painful headache were include in the study. Among the whole group, patients with seizure are more susceptible to harbour scanographic anomalies (60% and 79% respectively). 80% of them are positive for PCR and/or WB conducted in SCF. However for patients with seizure but with negative scanner, 37% and 56% were positive respectively for WB and PCR in SCF. This claims for an under-detection of lesions by standard scanner in field condition especially for recent infection. We are still registering patients in this study and isotype analysis of the WB is also in process to detect IgA, IgD and IgE anti- *T. solium* in SCF and blood of the patients. A score built on both epidemiological and biological data must be defining to improve the diagnostic of NCC. An overall analysis of these data will be presented.

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LONG-TERM SONOGRAPHIC FOLLOW-UP OF INACTIVE ECHINOCOCCAL CYSTS LOCALIZED TO THE LIVER

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The sonographic classification of echinococcal cysts proposed by the WHO Informal Working Group on Echinococcosis (WHO-IWGE) allows the distinction between active, transitional and inactive cysts, thus facilitating selection of treatment modalities. For uncomplicated, inactive cysts (CE4, CE5) in the liver, recent expert opinion recommends they should be left untreated and monitored, but no data exists on the safety and effectiveness of this approach. To fill this gap, we report our experience

with long-term sonographic monitoring of inactive cysts. Records of patients who presented at our clinic and were diagnosed with inactive echinococcal cysts of the liver were searched. Inclusion criteria were: 1) presence of cysts exclusively in inactive stage at the time of diagnosis; 2) follow-up with abdominal ultrasound performed every 6 or 12 months; and 3) minimal length of follow up of 24 months. For each patient, demographic details, characteristics of the cyst within the liver and complications and sonographic changes if they occurred during follow-up were obtained. From March 1994 to May 2011, 94 patients with exclusively inactive liver cysts were seen in our clinic with 41 meeting all inclusion criteria. Sixteen patients were male and 25 were female (mean age at time of diagnosis: 48 years, range: 14-86 years). They harbored a total of 55 cysts (of which 33 were CE4 type and 22 were CE5 type). The average cyst diameter was 52 mm. Twenty seven patients had 1 cyst each, 14 patients had 2 cysts each. 42 cysts were located in the right lobe, 4 in the left lobe and 9 in the fourth segment. The mean follow-up period was 78 months (range: 24-453 months) and in 40 patients (97.6%) the cysts remained in the inactive stage (reactivation occurred only in 1 patient). Our observations indicate that a proportion of cysts become completely inactive without any treatment and cysts that reached this stage are likely to remain inactive over time. Our data suggest that "Watch and Wait" may be a viable management option for uncomplicated inactive liver cysts.

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RE-EMERGENCE OF ECHINOCOCCOSIS IN NINGXIA HUI AUTONOMOUS REGION, PEOPLE'S REPUBLIC OF CHINA

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Human echinococcosis is a chronic debilitating disease causing severe morbidity and if poorly treated or left untreated has a mortality rate of approximately 90% within 10-15 years of diagnosis. Ningxia Hui Autonomous Region (NHAR), China is hyper-endemic for both cystic and alveolar echinococcosis (CE and AE) and has undergone great environmental change over the last decade as a result of deforestation due to rapid human population growth and more recently reforestation due to a policy shift. The policy to re-establish grassland has major implications for the transmission of both types of *Echinococcus* spp. This is likely to lead to an increasing human disease burden and to pose potentially severe problems for control. We undertook extensive investigations from 2001-2007 to update available epidemiological data and to monitor the transmission patterns of both *E. granulosus* and *E. multilocularis* in NHAR. The work of mass ultrasound surveys was conducted community-based (all age groups) in 2001-3, and serological screening of children aged 6-18 years in 26 villages in Xiji were undertaken and involved one cross-sectional survey of 861 randomly-selected children in 2001_3 and a repeat survey of 2600 randomly-selected children undertaken from 2006-7, including many of the same children. Ultrasound surveys clinical case detections have decreased markedly in NHAR over the last two decades, whereas sero-surveys of school- based (children), prevalence in 2006-7 was significantly greater than in 2001/3 ($P<0.001$). This is of great concern because it suggests that incidence of clinical cases is likely to rebound, with an increase in the burden of echinococcosis in coming decades. Although our previous spatial epidemiological study provided unprecedented insight into the environmental drivers of echinococcosis in Xiji, NHAR, it used land cover variables created at a single time-point that did not capture recent major landscape changes. Further studies are required to determine spatiotemporal variation in the landscape of NHAR.

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NOVEL INVASION MECHANISM EMPLOYED BY *TRYPANOSOMA CRUZI*

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Trypanosoma cruzi, causes Chagas disease. *T. cruzi* is capable of invading and replicating within a wide variety of cells. The molecular mechanism(s) of invasion and the regulatory pathways involved in this process has been the subject of intense investigation for many years. Although two models of invasion, which involve a lysosomal-dependent and a lysosomal-independent pathway have been described, the precise mechanism by which the parasite invades the host cell membrane barrier remains unknown. Herein, we report for the first time that host low density lipoprotein receptor (LDLr) is involved in host cell invasion by *T. cruzi* and the subsequent fusion of the parasitophorous vacuole with the host cell lysosomal compartment. The model suggested by this study unifies previous models of host cell invasion for this parasite. We demonstrate that *T. cruzi* directly binds to LDLr, and inhibition or disruption of LDLr significantly decreases parasite entry. We have also determined that this cross-linking triggers the accumulation of LDLr and phosphotidylinositol phosphates in coated pits, which initiates a signaling cascade that results in the recruitment of lysosomes. Our data is supported by the invasion studies carried out with siRNA LDLr knock-down cells. Studies with LDLr knock-out cells suggest that the parasite may bind to a different domain on LDLr other than the binding site for its natural ligand LDL. Additionally *T. cruzi* has demonstrated a high affinity for host LDL. The rate of invasion is directly correlated to the concentration of LDL in the medium. The infected mouse displayed an altered lipid profile. We observed a significant amount of LDL/cholesterol accumulation in the cells/tissues which may likely play a major role in the progression of chagasic heart disease. Our observations suggest that therapeutic strategies based on the interaction of *T. cruzi* and the LDLr pathway should be pursued as possible targets to modulate the consequences of infection.

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DEVELOPMENT OF *LEISHMANIA AMAZONENSIS* PARASITOPHOROUS VACUOLE IS INHIBITED BY TARGETING ER SNARES

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Leishmaniasis affects over 12 million people in 88 countries worldwide. *Leishmania* parasites reside in parasitophorous vacuoles (PV) whose biogenesis involves interactions with various host cell vesicles and organelles including the endoplasmic reticulum. A recent study confirmed that PVs interact continuously with the host cell ER; furthermore, the presence sec22b, on the PV membrane implicated the fusion with ER vesicles on PV biogenesis. Sec22b is an N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) that is localized in the ER. SNARE molecules mediate membrane fusion in eukaryotic cells via the formation of SNARE complexes by SNAREs on opposing membranes. In this study, we investigated the role of SNAREs that mediate the fusion of endoplasmic reticulum vesicles, on the distention of PVs and on parasite survival within host cells. Knockdown of sec22b and D12/p31 as well as the ER/Golgi SNARE syntaxin-5, but not the ER SNARE syntaxin 18, resulted in reduced PV growth, reduction in the number of infected cells and limited parasite replication. This was confirmed with the expression of dominant-negative forms of sec22b and D12/p31 (^tm-Sec22b and ^tm-D12/p31), which also resulted in reduced PV growth and limited parasite replication. These studies were extended by assessing the effect of the recently described small molecule, Retro-2, which inhibits syntaxin-5 function. Mouse macrophages infected with *L. amazonensis* and treated with Retro-2 two hours post-infection displayed a dose-dependent decrease in both PV size and parasite numbers. In *in vivo* studies, mice treated with Retro-2

immediately after infection or 3 weeks post-infection had significantly reduced footpad swelling as well as significantly reduced parasitemia over an 8 week infection course, as compared to control mice. These studies suggest that a strategy to inhibit PV maturation by targeting components of the host cell vesicle fusion machinery could be a feasible approach to control leishmaniasis.

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MECHANISMS OF CONTROL OF *TRYPANOSOMA CRUZI* AT THE INITIAL SITE OF PARASITE ENTRY

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Natural transmission of *Trypanosoma cruzi* frequently results from the entry of parasites present in the feces deposited by the insect vector, into breaks in the skin or mucosa. *T. cruzi* can often be detected in the blood stream during the acute phase of infection, prior to the development of adaptive immune response that limits parasites almost exclusively to muscle and adipose tissues during the chronic phase. To gain insights into the early events following *T. cruzi* infection in the skin, we studied the fate of fluorescently tagged *T. cruzi* delivered subcutaneously in the footpads or ears of C57BL/6 mice, using *in vivo* imaging and flow cytometry during the first week after infection. We demonstrate that the majority of parasites introduced into the skin initially proliferate at this infection site. Expansion of the infecting parasites continues at the site of inoculation until 8-10 days post infection when the parasite load, measured by fluorescence intensity, drops below the limit of detection. The decrease in the parasite numbers 8-10 days post-infection is dependent on the presence of an intact T cell compartment and on the ability of hosts to produce IFN- γ. Mice deficient in CD4+ or CD8+ cells or in the expression of lymphotoxin-α experience delayed control and higher levels of parasite growth at the infection site. Flow cytometric analysis shows that many of the parasite-containing cells at the site of inoculation display a surface phenotype compatible with myeloid dendritic cells (CD11c+CD11b+). However these parasite-containing myeloid dendritic cells are not activated to produce IL-12 expression. Our results suggest that *T. cruzi* preferentially expands at the site of inoculation, dispersing from this site prior to or as immune mechanisms begin to limit this local parasite growth. Future studies will determine the extent of parasite control at the initial site (whether partial or complete) and why the mechanisms regulating parasite numbers at the site of infection do not prevent parasite expansion to other sites in the body.

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TREATMENT OF AN INTRACELLULAR PATHOGEN BY TARGETING PI3KΓ SIGNALING IN THE HOST

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The Leishmaniasis are a group of diseases resulting from infection with protozoan parasites of the genus Leishmania. Cutaneous leishmaniasisis (CL) the most widespread form of disease and is common throughout Central and South America, Africa, India, and Southwest Asia. CL results in the formation of well defined, localized lesions that generally self-resolve without need for treatment. However, some patients experience progressive disease and develop non-healing chronic infections. Problems associated with enhanced drug resistance by parasites and increased patient-unresponsiveness towards standard chemotherapeutics has become a significant concern for health officials' worldwide. With 350 million people currently living in areas of active parasite transmission, the need for effective therapeutics to treat infection is considered a primary goal of The World Health Organization. The Phosphoinositide 3-Kinases (PI3Ks) are a large family of evolutionarily conserved protein kinases involved in intracellular signal transduction. Studies using broad-spectrum PI3K inhibitors such as wortmannin and LY294002 have shown that Class I PI3Ks are involved in phagocytosis and in mediating entry of parasites such as *Trypanosoma cruzi* into host cells however, the precise role of each Class I PI3K isoform remains unclear. PI3Kγ is expressed primarily by immune cells and has been shown to play a critical role in chemotaxis by controlling actin cytoskeletal reorganization. We have characterized a role for PI3Kγ in CL caused by *Leishmania mexicana*. Using a PI3Kγ-selective inhibitor, AS- 605240, and PI3Kγ-deficient mice, we demonstrate that PI3Kγ contributes to the pathogenesis of CL by mediating recruitment of phagocytes and regulatory T cells to the site of infection and by facilitating parasite entry into phagocytes. Furthermore, we demonstrate that AS-605240 is as effective as standard anti-leishmanial chemotherapy with sodium stibogluconate in limiting parasite growth after infection. These findings reveal a novel role for PI3Kγ in *L. mexicana* invasion and establishment of chronic infection, and demonstrate that therapeutic targeting of host pathways involved in establishment of infection is a viable treatment strategy for treating CL caused by *L. mexicana* and possibly other intracellular pathogens of phagocytes.

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SUBVERSION OF INNATE IMMUNE SIGNALS BY *SCHISTOSOMA MANSONI* PERMITS WORM DEVELOPMENT

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Blood flukes of the genus *Schistosoma* infect 200 million people. As a result of host parasite co-evolution, *S. mansoni* has evolved to exploit host immune factors as signals to coordinate its own development within the host. Worms fail to develop normally in $RAG^{-/-}$ mice that lack all T and B cells, while development is restored when $CD4^+$ T cells are transferred into $RAG^{-/-}$ mice, suggesting that $CD4^+$ T cells play a role in regulating parasite development. Recent findings suggest the role of $CD4^+$ T cells in this process is indirect, limited to provision of T cell help for innate responses which, in turn, facilitate parasite development. In support of this hypothesis, we have found that direct activation innate responses in $RAG^{-/-}$ mice by lipopolysaccharide (LPS) administration also restores worm development, indicating that innate immune signals are sufficient for parasite development to proceed normally. In addition to LPS, a variety of other pathogen-associated molecular patterns (PAMPs) that activate the MyD88 pathway via toll-like receptors (TLRs) also restore parasite development. Interestingly, TRIF-dependent signaling failed to restore worm development in $RAG^{-/-}$ mice, suggesting a parasite requirement for MyD88-dependent responses. However, high levels of PAMPS are not present during the normal course of a *S. mansoni* infection, suggesting that MyD88 responses must be initiated by other means under normal circumstances. We therefore hypothesize that, during schistosome infection, endogenous danger-associated molecular patterns (DAMPs) induce the innate responses required for parasite development, following their release by damaged host cells such as hepatocytes. In support of this, we show that stimulation of the NALP3 inflammasome, a MyD88-dependent sensor of endogenous DAMPs, restores worm development in $RAG^{-/-}$ mice. Current research efforts are focused on dissecting the MyD88-linked signaling events that influence schistosome development. Elucidation of the innate immune signals that control schistosome development may help in the development of new drug targets and vaccine strategies.

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FECAL SHEDDING OF NON-TYPHOID *SALMONELLA* SPECIES IN DAIRY CATTLE AND THEIR ATTENDANTS IN ALEXANDRIA, EGYPT

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Salmonella infections in dairy cattle continue to be a major worldwide problem. Substantial economic losses were manifested through mortality and poor growth of infected animals as well as the hazard of transmission to humans either through food chain or direct animal contact.Our objective was the isolation and identification of Salmonella spp. shed in feces of dairy cattle and their attendants,together with the determination of their serotypes and antimicrobial susceptibility patterns. Fecal samples were cultured on non selective pre-enrichment broths,and selective agar media.Serotyping of *Salmonella* spp.isolates was performed by slide agglutination tests with antisera and then screened for their antibiotic susceptibility by single disc diffusion method.Seven Salmonella spp. were isolated from examined dairy cattles while no one was isolated from any of the examined attendants. *Salmonella* isolates were classified as serogroups B, C1, D1 and E1, with C1 as the most commonly observed serogroup (57.1%). Five different *Salmonella* serotypes were identified (Typhimurium, Anatum, Concord, Montevideo and Enteritidis). The 7 isolated *Salmonella* spp. showed no resistance to all tested antimicrobial agents except for trimethoprim-sulphamethoxazole and gentamycin.

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POPULATION STRUCTURE OF ENTEROAGGREGATIVE *ESCHERICHIA COLI*

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Enteroaggregative *Escherichia coli* (EAEC) is a major cause of infectious diarrhoeal disease. The ICDDR, Bangladesh has a major research programme looking at the impact of EAEC and recent studies in Southern Nigeria have shown it to be one of the most common bacterial pathogens from children with diarrhoea. The true burden of disease is unknown because there is a lack of markers associated with diarrhoeagenic strains of EAEC. Attempts to find selective markers to delineate disease causing EAEC have led to the use of the plasmid-borne anti-aggregative transporter (*aat*) gene, however studies have shown *aat*-positive *E. coli* with to be present in both cases and healthy controls. The present study aims to delineate disease causing sub-groups of EAEC. Using sequence typing of the background strains as well as virulence factor detection appropriate diagnostics will be developed. One hundred and twenty five EAEC isolates from Nigeria and 83 isolates from Bangladesh (defined by the presence of *aat* or HEp-2 EAEC phenotype) were genotyped using Multi-Locus Sequencing Typing (MLST) and the sequence types from cases and controls were compared. This will allow the association of virulence factors with the background of the EAEC strains and so the definition of "pathotypes". Of the Nigerian isolates 133 are from 98 sequence types (STs), of which 48.3% clustered into two main clonal complexes. One of these complexes, ST10, showed significant association with disease in Nigerian children less than one year of age. In Bangladesh 52 different STs were delineated of which 27 were not present in the current database. Complex associations were seen among clonal groupings of EAEC and the ability to cause disease: ST38 was associated with disease and ST295 was associated mainly with controls. Enteroaggregative *Escherichia coli* can be redefined by their ability to cause disease using sequencing and population structure analysis which will facilitate pathogenesis research and the development of an accurate diagnostic test.

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DIARRHEAGENIC *ESCHERICHIA COLI* PHYLOGENY AND ITS ASSOCIATION WITH DIARRHEA

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Diarrheagenic *Escherichia coli* (DEC) are important causes of diarrhea in children, however their phylogenetic group associations are not well defined. Traditionally phylogenetic groups A and B1 are more associated with non-severe illness while B2 and D are associated with more severe illness. The aim of this study was to examine the association of DEC phylogeny with diarrhea severity in children. The phylogenetic group of 369 *E. coli* strains isolated from Peruvian children <1 year of age was determined by a triplex PCR (Clermont). DEC were defined by multiplex real time PCR for diarrhea associated virulence genes. The clinical data on 127 strains isolated from diarrhea episodes was analyzed by a modified Vesikari severity score (episode duration, maximum number of stools per day and per episode, dehydratation, blood in stools, maximum number of emesis per day and per episode and fever). DEC isolated from healthy control children (n= 94) and DEC isolated from infants with diarrhea (n=201) were phylogenetically classified as A (50% and 37%), D (21% and 34%), B1 (26% and 22%) and B2 (3% and 7%) respectively. DEC-control strains were more associated with A group while DEC-diarrhea strains more associated with D group ($p<0.05$). Commensal E. coli (n=74) were more associated with A (35%) and D (38%) groups. Commensals also had a high prevalence of B2 group (16%) unlike both DEC groups ($p<0.05$). Both DEC groups were more associated with B1 group than commensals (11%) ($p<0.05$). For the 127 diarrhea strains with clinical data, no differences were found between the phylogenetic groups and the severity score. However among non- severe groups (A-B1) and severe groups (B2-D), there was a tendency ($p=0.06$) for B2-D to be associated with persistent diarrhea ($\geq$ 14 days). In summary, among DEC, diarrhea strains were more associated with group D than control samples. There was a tendency for persistent diarrhea strains to belong to B2 and D.

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PREVALENCE OF ENTERIC PATHOGENS IN KENYA

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Acute diarrhea remains a major public health problem in developing countries and is responsible for nearly 2.5 million deaths of children under the age of 5. Of the enteric studies conducted in Kenya over the past 20 years, the majority of the findings have been clustered around bacterial outbreaks with a few individual studies reporting on parasitic and viral causes. Accurate epidemiologic information on acute diarrheal illness in this region will be critical for augmenting effective travelers' diarrhea management policies**.** Stool samples from age-matched cases (subjects with acute diarrhea; 3 or more loose stools in a 24 hr period) and controls (no acute diarrhea symptoms) were collected from several district hospitals in western and central Kenya. Diagnostic microscopy was used to identify helminth ova and protozoan cysts. Isolation and identification of bacterial pathogens was by conventional microbiological methodologies while detection of rotavirus was done using Rotaclone kits. Antibiotic susceptibility of bacterial isolates was ascertained using the Microscan Walkaway 40 Plus. On microbiological examination, enteric pathogenic agents were detected from 207 (28.3%) of 731 fecal specimens collected (387 cases and 344 controls). The agents were bacterial: 23.75% (*Salmonella spp, Shigella spp, Campylobacter jejuni*, *Yersinia enterocolitica*, and enterotoxigenic *Escherichia coli*), rotavirus: 26.5% and parasitic: 49.75% (*Giardia lamblia, Entamoeba histolytica, Cryptosporidium parvum, Ascaris lumbricoides, Schistosoma mansoni, Strongyloides stercoralis*, *and Ancyclostoma duodenale/Necator americanus*) respectively. Of

the bacterial pathogens, *Shigella* spp was the most common while *G. lamblia* was the most common parasite. Antibiotic susceptibility testing of the *Salmonella* and *Shigella* isolates showed that there is multidrug resistance (MDR) among 35 - 80% of the isolates to common antibiotics used for treatment to include tetracycline, amoxicillin, chloramphenicol, trimethoprim sulfa, and amoxicillin-clavulanic acid. Future surveillance will be established in eastern and northern Kenya.

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MEMORY B CELL RESPONSES AND OTHER ACUTE IMMUNE RESPONSES IN BANGLADESHI CHILDREN AFTER INTAKE OF ORAL KILLED CHOLERA VACCINE, AND COMPARISON WITH RESPONSES FOLLOWING NATURAL CHOLERA INFECTION

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Young children bear a large burden of cholera caused by *Vibrio cholerae* O1 globally. Unfortunately, cholera vaccine studies have demonstrated lower long-term protective efficacy in children under 5 years compared to older children and adults. The mechanism behind this discrepancy remains to be elucidated. Memory B cell (MBC) responses may correlate with duration of protection following infection and vaccination. We report a comparison of immune responses in young children (3-5 years of age; *n*=20) and older children (6-14 years of age; *n*=20) given two doses of an oral killed cholera vaccine (Dukoral, Crucell) 14 days apart. We assessed responses before vaccination, at 3 days after the first dose, and at 7 and 28 days after the second dose. We found that the two age groups had comparable vibriocidal antibody responses on all study days. Older children had higher plasma IgG responses to cholera toxin B subunit (CtxB) than young children at day 7 (P=0.045) and day 28 (P=0.003). Older children trended towards higher MBC responses to CtxB than younger children at study day 28 for both IgG (P=0.09) and IgA (P=0.12) responses. We also compared immune responses of the vaccinees to age-matched patients with cholera due to *V. cholerae* O1 Ogawa infection. We found that for all children, vaccinees had significantly lower day 7 responses to vibriocidal antibody (P<0.001) than patients. We also found that younger children had significantly lower plasma IgG (P=0.008) and IgA (P=0.001) day 7 responses to lipopolysaccharide (LPS) than age-matched patients. Memory B cell IgG and IgA responses targeting LPS and CtxB were detected in all vaccine and patient cohorts, although fold-change increases in responses were most pronounced in patients following wild type disease. Our findings suggest that immune responses following oral-killed cholera vaccination are generally comparable in younger and older children, but that these responses are lower than that observed following wild type disease. These findings may explain, in part, the lower efficacy of cholera vaccine in children.

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COMPLETE SEQUENCE OF THE LARGE VIRULENCE PLASMID OF ENTEROAGGREGATIVE *ESCHERICHIA COLI* STRAIN 60A

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Enteroaggregative *Escherichia coli* (EAEC) are an important cause of childhood diarrhea in developing countries. EAEC are defined by a characteristic aggregative pattern of adherence but, as we have recently demonstrated, this phenotype is convergent. Multiple EAEC lineages warrant investigation, prompting our evaluation of EAEC strain 60A, originally isolated from a child with diarrhea in Mexico. We marked the large virulence plasmid of strain 60A with a chloramphenicol resistance gene and found it sufficient to confer autoaggregation, biofilm formation and virulence in the nematode *Caenorhabditis elegans* on non-pathogenic *E. coli*. Complete sequence and preliminary annotation of the plasmid reveals that it is a 90,229bp molecule with a G+C content of 47.1%. The plasmid carries genes predicted to encode aggregative adherence fimbriae, the Aap dispersin and its secretion system, the AggR regulator and a serine protease autotransporter precursor. We used a transposon mutagenesis library to validate contributions of some of these genes to biofilm formation and *C. elegans* virulence. Also identified on the plasmid were conjugative transfer genes, plasmid maintenance genes, open reading frames of likely bacteriophage origin and multiple insertion elements, most flanking putative virulence genes. Some, but not all of these loci are found on other sequenced EAEC plasmids, but the 60A plasmid lacks toxin genes that have been reported from other EAEC. The data suggest that EAEC plasmids are virulence gene mosaics and this project presents a strategy for functional annotation of small genomes that could be applied to other virulence plasmids.

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MALNUTRITION AND DIARRHEAL DISEASES IN A COHORT STUDY IN THE BRAZIL SITE

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Diarrheal diseases and malnutrition are associated with over a half of children <5yo deaths (5.6 million) where new biomarkers and interventions to prevent or treat these diseases are needed to decrease the mortality and importantly the morbidity associated with these diseases. This study was undertaken to identify the risk factors in children associated with malnutrition, diarrheal diseases, impaired gut function, vaccine response, impaired development and cognitive functions in prospective longitudinal study in Northeast Brazil. The prospective longitudinal cohort design will enroll 200 children at birth to be followed twice weekly for 24 months for all disease morbidity and for microbiological, clinical, nutritional, gut function and cognitive function assessments. Up to 06Apr11 the cohort study screened 100 pregnant women who signed the consent form and 100% were eligible with none refused. A total of 100 children were enrolled, four were lost to follow-up (3 moved and 1 refused the study protocol) and none died. The mean (SD) age of their entry was 9 days (4.5), all were < 17 days old and 50% were male. The mean birth weight (Kg; SD) was 3.9 ± 0.52. The mean z-scores (SD) for weight-for-age (WAZ), height-for-age (HAZ) and weight-for-height (WHZ) during this period of observations were 0.456 (1.054), -0.222 (1.091) and 0.878 (1.159), respectively. The mean (days; SD) for exclusive breastfeeding, mixed breastfeeding and non-breastfeeding were: 19.3 (13.24), 33.4 (19.98) and 144.2 (72.53), respectively. A total of 418 stool samples were collected and 3 (0.7%) were from diarrheal illnesses. The lactulose:mannitol ratio was abnormal in 82% (50/61; abnormal LM ratio =or> 0.0864) of the tests performed for the children enrolled so far. In conclusion, with now approximately 50% of the total enrollment plan for the cohort study, within the first 6 months of follow-up, most children had low diarrhea rates, normal nutritional status but are already exhibiting changes in intestinal barrier function where followup growth will be of great interest.

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LABORATORY SURVEILLANCE OF *VIBRIO CHOLERAE* STRAINS FROM HAITI, 2010-2011

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As of May 3, 2011, there have been 293,470 cases and 4,954 deaths from the cholera outbreak in Haiti. LNSP and CDC laboratories characterized 198 *Vibrio cholerae* isolates collected between October 2010 and March 2011 from 10 Haitian departments to monitor genetic variation and antimicrobial resistance. Serogroup and serotype were determined by agglutination with specific antisera. Antimicrobial susceptibility was determined by disk diffusion and broth microdilution. Genetic analyses included PCR detection of species (*owpW*, *toxR*), biotype (*tcpA*), and cholera toxin (*ctxA*) genes. A hierarchical sampling scheme was used in further strain characterization; 152 isolates were characterized by pulsed-field gel electrophoresis (PFGE) with *Sfi*I and *Not*I enzymes. Based on PFGE results complete DNA sequence analyses of *ctx*AB and *tcp*A genes were determined for 85 *ctx* positive strains. One hundred and ninety isolates were identified as toxigenic *V. cholerae*, serogroup O1, serotype Ogawa, biotype El Tor. All isolates were resistant to furazolidone, nalidixic acid, streptomycin, sulfisoxazole and trimethoprim-sulfamethoxazole. Fifteen PFGE patterns were identified with one predominant pattern combination representing 78% of the collection. Seven new variant patterns (4.5%) were observed one month after the outbreak was first identified. The *ctxAB* and *tcp*A sequences were identical for all isolates; the *ctxB* gene matched the B7 allele first observed in a 2007 outbreak isolate in Orissa, India. Eight isolates were identified as nontoxigenic, non-O1, non-O139 isolates with less than 80% similarity to the dominant Haiti outbreak strain by PFGE. Minor variations in PFGE patterns of the outbreak strain have arisen but changes in antimicrobial resistance have not been observed. The nontoxigenic *V. cholerae* isolates are genetically unrelated to the outbreak strain. Routine surveillance using a combination of phenotypic and genotypic methods is critical for cholera monitoring to identify epidemic cholera from sporadic cases.

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RISK FACTORS FOR THE DEVELOPMENT OF ILEAL PERFORATION AMONG PATIENTS AT KAGANDO HOSPITAL, KASESE, UGANDA

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Perforation of the ileum is a debilitating complication of typhoid fever, a water-born bacterial disease that is most commonly found in areas with poor clean water access and sanitation. Demographics of ileal perforation patients at Kagando Hospital in Kagando, Uganda were gathered retrospectively from hospital records. The distribution of cases was determined using geographic information technology (GIS), and a case-control study was used to discover risk factors. Interviews of 93 previous patients with a confirmed ileal perforation were conducted, and a control subject with no previous history of typhoid or perforation was interviewed for each patient matched by sex, age and village of residence. Standardized interviews were completed to assess the patient's previous medical history, level of clean water and sanitation access, and socioeconomic status. From January 2004-September 2010, 409 patients with confirmed cases of ileal perforation were admitted to Kagando Hospital. Of the 336 with an adequate medical history for further analysis, 198 (58.9%) were male with a mean age of 24.4 (range: 1-95). A analysis of the geographic distribution of patient households shows a more dense concentration in the western side of the hospital catchment area. Failure to treat drinking water (χ^2= 9.52, p= 0.02), having a family member with typhoid symptoms (χ^2= 3.71, p= 0.05), having a home construction of thatch (χ^2= 20.25, p= <0.001), low levels of literacy in the household (F= 9.67, p= 0.002), and having family members who did not complete secondary school (χ^2= 5.74, p= 0.02) were associated with ileal perforation. Results suggest that perforation patients do not have access to clean, reliable water sources, are at a lower socioeconomic level, and are less educated than patients without a history of an ileal perforation, which has significant implications for future hospital initiatives and public health policy in the area. Additionally, the results reveal the need to address the ecological, social, and biological precursors for a holistic response.

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BURDEN OF *AEROMONAS HYDROPHILA*-ASSOCIATED DIARRHEA AMONG CHILDREN LESS THAN TWO YEARS IN A RURAL EGYPTIAN COMMUNITY

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Aeromonas hydrophila is increasingly recognized as a cause of diarrhea. However, limited data are available on the prevalence and severity of *A. hydrophila*-associated diarrhea in the Middle East and Africa. Between 2004 and 2007, children living in the Nile Delta of Egypt were enrolled at birth and visited at home twice weekly for two years. A stool sample was collected every two weeks and whenever a child was reported to have diarrhea. Samples were cultured for bacterial pathogens using standard techniques. ELISA was utilized to detect enteric viruses as well as *Cryptosporidium spp.* Of the 348 enrolled children, *A. hydrophila* was isolated from the stool of 79 (22.7%). Thirty-three (9%) children were asymptomatic while 46 (13%) had diarrhea at the time of stool collection. *A. hydrophila* was the only organism isolated in 20 (6%) children with diarrhea. The probability of *A. hydrophila* infection to be associated with diarrhea was 0.72 and increased to 0.96 when no other pathogen was detected. The incidence rate (IR) of *A. hydrophila*-associated diarrhea (sole pathogen) was 0.07episode/child/year. The peak of diarrhea incidence occurred in the second six months of life (IR 0.13 episode/child/year). Ninety percent of episodes occurred mainly in the warm season (18/20) with 55% characterized by fever in (11/20) and 15% characterized by vomiting (3/20). Of 34 episodes of any-cause diarrhea associated with dehydration in the study subjects, only one episode was associated with *Aeromonas* infection, while 18 were associated with ETEC, 11 with rotavirus, and one case each with *Campylobacter* spp. and *Shigella* spp. The median duration of *A. hydrophilia*-associated diarrhea was four days (IQR 2-5 days) and the median number of loose stools in any day was six (IQR 4-7). Susceptibility testing demonstrated that 0.8% of isolates were sensitive to ampicillin, 25% to cephalothin, and 96% to ciprofloxacin. This study demonstrates that *A. hydrophila* is associated with diarrhea that is less severe compared to cases due to other enteric pathogens isolated among children living in the Nile Delta.

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ACUTE PHASE PROTEINS, BREAST FEEDING AND DIARRHEAL DISEASES IN A CASE CONTROL STUDY IN NORTHEASTERN BRAZIL

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Intestinal inflammation has been associated with diarrheal diseases and enteropathy where new markers like acute phase proteins are potentially useful to evaluate intestinal barrier damage. This study was undertaken to evaluate the influence of breast feeding on lactoferrin, neopterin, alpha1-antitrypsin and myeloperoxidase acute phase protein markers of intestinal inflammation in a case control study in the Northeast of Brazil. The design was a study of 1,200 children (600 cases and 600 age and neighborhood matched controls), age 3-36 months, with a cross section study for the disease morbidity, microbiological, clinical, nutritional and gut function stool samples ELISAs evaluation for acute phase proteins: lactoferrin, neopterin, alpha1-antitrypsin and myeloperoxidase. Cases were defined as diarrhea with more than three liquid stools in the last 24 hours and controls were without history of diarrhea in the last two weeks. After the mothers or caregivers signed the consent form, we evaluated the first 200 children, 102 cases and 98 controls, with mean age of 15.27 ± 9.212 and 20.68 ± 8.767 months old, respectively. Males were 51% in cases and 50% in the controls. Less than 10% of all children had exclusive breast feeding and 60% (61/102) and 40% (39/98) had mixed breast feeding for cases and controls, respectively. Lactoferrin was significantly higher in all breastfed compared to non-breastfed children, as well as breastfed cases or controls compared to their respective non-breastfed controls ($p<0.001$; unpaired t test). Alpha1-antitrypsin, neopterin and myeloperoxidase had also similar significant results when breastfed were compared to non-breastfed children's stool samples. In conclusion, the data suggest that children on breast feeding can over estimate positive results for lactoferrin, neopterin, alpha1-antitrypsin and myeloperoxidase stool markers of inflammatory acute phase proteins. New and further studies of potential quantitative inflammatory markers are warranted to avoid the influence of breast feeding on those markers.

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ANTIMICROBIAL RESISTANCE TRENDS OF *CAMPYLOBACTER SPP*. IN PERU

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Campylobacter jejuni and related species are food-borne zoonotic pathogens with a major role in the etiology of human bacterial enterocolitis globally. Emerging antimicrobial resistance in *C.jejuni* has been described in both developed and developing world settings, with important therapeutic implications. We describe here trends in fluroquinolone and macrolide resistance over a 10-year period in multiple regions of Peru. Antimicrobial susceptibilities of 4652 *Campylobacter spp.* stool isolates from three regions of Peru over a 10-year period between 2001 and 2010 were reviewed. Isolation and identification were performed by standard microbiological techniques. Susceptibilities to ciprofloxacin, azithromycin and erythromycin were determined by disk diffusion methods as per CSLI guidelines. Susceptibilities of isolates from medical centres in Lima (n = 3419) Iquitos (n = 625) and Cusco (n = 608) were reviewed**.** The majority (82%) of all isolates were identified as *C. jejuni*, followed by *C. coli* (11%) and other *Campylobacter spp.* (7%). The prevalence of ciprofloxacin resistance among isolates from Lima increased from 46% to 92% over a ten year period. A similar increase was seen in the Cusco region from 50% to 86%. In isolates from Iquitos, ciprofloxacin resistance prevalence increased from 19% to 38%. Macrolide resistance remained low in Cusco and Lima with only 1% of Lima isolates and 2% of Cusco isolates observed as azithromycin resistant; Erythromycin resistance was noted in 1% of Lima isolates and none of the Cusco isolates. In contrast, azithromycin resistance increased from 3% to 14% of Iquitos isolates over ten years and erythromycin resistance increased from 3% to 17%. These results have significant therapeutic implications for the empirical management of enterocolitis in Peru. Ongoing resistance surveillance in all regions is essential to guide appropriate antimicrobial use.

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FIVE *ESCHERICHIA COLI* SUB-TYPES INFECTION IN PROBE-BASED DETECTION WITH LUMINEX BEADS IN A CASE CONTROL STUDY OF CHILDREN IN NORTHEASTERN BRAZIL

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Several bacteria can cause diarrheal diseases and polymerase chain reaction (PCR) assays are increasingly being used on fecal DNA samples for enhanced specificity and sensitivity detection. Substitution of PCR-based assays for classical clinical microbiology tests are a foreseeable aim for the near future. We examined a new developed multiplexed assay for simultaneous detection of major E. coli sub-types pathogens in a case control study of diarrheal diseases. The design was a study of 1,200 children (600 cases and 600 age and neighborhood matched controls), age 3-36 months. Cases were defined as diarrhea with more than three liquid stools in the last 24 hours and controls were without history of diarrhea in the last two weeks. A simple protocol combining a one-step multiplex PCR with microsphere-based fluorescence detection was used for shiga producing E. coli (STEC: Stx 1/2 with eae), enteropathogenic E. coli (typical: eae and bfpA and atypical: eae alone; EPEC), enteroaggregative E. coli (aaiC and aatA; EAEC) and enterotoxigenic E. coli (ST or LT; ETEC). After the mothers or caregivers signed the consent form, we evaluated the first 200 children, 102 cases and 98 controls, with mean age of 15.3 ± 9.21 and 20.7 ± 8.77 months old, respectively. Males were 51% in cases and 50% in the controls. Less than 10% of all children had exclusive breast feeding and 60% (61/102) and 40% (39/98) had mixed breast feeding for cases and controls, respectively. Mean duration of diarrhea was 3.3 ± 0.91 days, 25% (25/101) were severity (at least one day with =>5 liquid stools/24 hours) and 15% (15/101) had moderated dehydration. Typical EPEC (11%; 11/97 vs 2%; 2/97) and shiga producing E. coli (11%; 11/97 vs 3%; 3/97) were significantly associated with diarrhea cases compared to control children ($p<0.001$; Pearson Chi-Square test). In conclusion, the data suggest that children with acute diarrhea are associated with typical EPEC and shiga producing E. coli. These results are also consistent with single and multiplex PCRs and this one-step nucleic acid-based luminex assay enables rapid detection of the major E. coli sub-types infections.

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DETECTION OF *PSEUDOMONAS AERUGINOSA* STRAINS PRODUCING TYPE-VIM METALLOBETALACTAMASES ISOLATED FROM HEALTH CENTERS OF NORTHEASTERN VENEZUELA

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Metallobetalactamase-producing *Pseudomonas aeruginosa* strains have been implicated in hospital-acquired outbreaks in different parts of the world and the increase in MBL's prevalence is mostly due to clonal dispersion of the strains and horizontal gene-transfer through mobile genetic elements. To determine the prevalence of VIM-type MBL gene, we evaluated a total of 311 *P. aeruginosa* strains from central hospitals in Cumana (n=137), Carupano (n=77), both in Sucre state, and Puerto La Cruz-Barcelona (PLC-B, n=97), Anzoategui state. Antimicrobial susceptibility of the strains isolated from a variety of samples and from different hospital's units, was assessed using Kirby-Bauer disk-diffusion assay, for the betalactams ceftazidime, cefepime, aztreonam, imipenem y meropenem. We also determined the presence of Metallobetalactamases (MBLs) using the double-disc synergy test with Imipenem/Meroperem and EDTA-SMA. Those strains showing the presence of MBLs were used to amplify a 382 pb fragment by PCR using primers specific for VIM-type MBL gene. Higher phenotypic resistance was seen in the Cumana strains for ceftazidime (10.9%), imipenem (32.1%) and meropenem (29.9%), followed by PLC-B (3.1, 11.3, 12.4%, respectively) and Carupano (2.6, 5.2, 7.8%, respectively). However, cefepime and aztreonam resistance showed very low frequencies. We also found higher frequency of MBL-producing strains in Cumana (25.5%), compared to PLC-B (10.3%) and Carupano (9.1%). All the MBL-producing strains showed amplification of the VIM gene. This study demonstrates the high prevalence of VIM-type MBL-producing *P. aeruginosa* strains in the Northeastern region, especially in Cumana central Hospital. These findings are very relevant for the epidemiology of bacterial resistance in the region and should serve as an alert for the health authorities to design campaigns to reduce the impact and spreading of resistant strains and mobile genetic elements that can increase the health risk of the patients, the length of their hospital stay and the costs of their treatment.

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FORMATIVE ASSESSMENT OF ACCEPTABILITY OF TYPHOID VACCINE IN NENO DISTRICT, MALAWI

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Typhoid fever affects an estimated 21 million people annually and causes 200,000 deaths worldwide. In June 2009, an outbreak was detected in southwestern Malawi. Despite efforts to implement improved water and sanitation measures to prevent transmission, new cases continued to be confirmed. In response, other strategies to control the outbreak, including the introduction of typhoid vaccine, were considered. Although typhoid fever outbreaks are common in sub-Saharan Africa, the acceptability of typhoid vaccine as a control measure has not been previously assessed. We carried out an investigation in August and September 2010 to examine factors associated with the acceptability of a typhoid vaccine in Neno District where the outbreak was ongoing. The investigation employed qualitative methods, including freelisting exercises (n = 31 participants), key informant (n = 8) and in-depth interviews (n = 20), and group discussions (n = 5). Respondents associated illness with exposure to "bad wind," and transmission was believed to be airborne. Community members considered typhoid to be extremely dangerous due to its rapid onset and spread, the peculiar signs and symptoms such as an inability to walk and the mental disturbances it produced, the number of fatalities and speed with which they occurred, and the perception that it was highly contagious. Respondents were skeptical about the effectiveness of water and sanitation interventions, indicating they had been following the same hygiene practices for years without experiencing typhoid, new cases continued despite these interventions, and the strategies conflicted with local disease models. Respondents generally understood the purpose of vaccination and believed in its ability to protect people from illnesses. The perceived severity of typhoid, continued concern of risk of exposure to disease, uncertainty about the effectiveness of implemented preventive measures, and widespread belief in the efficacy of vaccines in preventing disease resulted in an overwhelming interest in receiving a typhoid vaccine during an outbreak.

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GENOTYPIC AND PHENOTYPIC CHARACTERIZATION OF ENTEROTOXIGENIC *ESCHERICHIA COLI* (ETEC) STRAINS ISOLATED IN PERÚ

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Enterotoxigenic *Escherichia coli* (ETEC) are important etiological agents of diarrheal disease, especially in developing countries. Various strains of ETEC can be distinguished using genotypic and phenotypic markers. In this study, 45 ETEC strains isolated over a three year period from clinical diarrhea samples collected in the coastal (Lima, Piura, and Pisco), Andean (Cusco) and Amazonian (Puerto Maldonado and Iquitos) regions of Peru were evaluated for the prevalence and distribution of heat-labile (LT) or heat-stabile (ST) enterotoxins, colonization factors (CFs), and genotypes. Stool was cultured from both pediatric and adult patients reporting with acute diarrheal to local regional hospitals. Five lactose fermenting colonies per culture were selected presumptive ETEC identification by PCR for LT and ST. Enterotoxins were confirmed using a GM1-based enzyme-linked immunosorbent assay. Confirmed strains were further tested for CFs phenotypes against a panel of 21 monoclonal antibodies by a dot-blot assay. The genetic diversity among the strains was investigated by randomly amplified polymorphic DNA (RAPD) analysis on genomic extracts using primers 1254 and 1290. Twenty-five of the 45 strains evaluated (55.5%) harbored the ST but not the LT genes (ST+/LT-), 26.7% were ST-/LT+ and 17.8% were ST+/LT+. A CF was identified in 24 strains (53.3%). The most prevalent CFs detected were CS1 (found in 62.5%), CS12 (20,8%), CS3 (12.5%), CFA/I (12.5%) and CS6 (8.3%). 20 unique RAPD profiles (7 A-type and 8 B-type) were found, with A2 B1 the predominant profile found in 26.6% of the strains.This study is the first investigation of genotyping of strains of ETEC in Peru. The information generated is of epidemiological importance and for determining coverage in Peru of investigational ETEC vaccines.

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A NORTHEAST BRAZIL REGIONAL BASIC DIET PROMOTES SMALL INTESTINAL MUCOSAL ATROPHY, DEFECTS IN BARRIER FUNCTION AND BACTERIAL TRANSLOCATION IN WEANLING MICE

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Environmental enteropathy (EE) is hypothesized to be an intestinal manifestation of the malnutrition-infection cycle in children who lack access to safe water and sanitation. EE pathogenesis remains poorly understood, however current evidence suggests that the weaning of infants to marginal diets promotes defects in intestinal epithelial structure and function that predispose to enteric infections, malabsorption, translocation of gut bacteria to the blood, and subsequent systemic immune activation. We sought to determine the intestinal effects of such a diet in weanling mice and compare these with human EE. Dams of 10-day-old C57BL/6 pups were randomized to a Northeast Brazil regional basic diet (RBD, 5% fat, 7% protein, and 88% carbohydrate) or a balanced, isocaloric control diet (15% fat, 20% protein, and 65% carbohydrate). Pups were weaned to their dams' diet on day of life 21 and monitored for growth. At 6 weeks of age, weanlings were sacrificed and jejunal tissue was collected for Ussing chamber analysis of transmucosal resistance and permeability; morphometry; and immunohistochemical staining for epithelial proliferation and apoptosis. Mesenteric lymph nodes were harvested and cultured to assess bacterial translocation. Pups of RBD-fed dams showed reductions in weight and tail length relative to well-nourished controls. Jejunal specimens from RBD-fed weanlings exhibited decreased villous height and crypt depth, decreased transmucosal resistance, increased permeability to FITC-dextran, and decreased epithelial proliferation and increased epithelial apoptosis (as measured by BrdU and cleaved caspase-3 staining, respectively). Mesenteric lymph nodes from RBD-fed mice showed a higher bacterial load (cfu/mg). No significant intestinal inflammation was seen in either RBD-fed mice or controls. In conclusion, the regional basic diet induces failure to thrive and intestinal mucosal derangements in mice that mimic key features of human environmental enteropathy. Additional mouse studies are needed to model the inflammatory component of human environmental enteropathy and further assess the role of bacterial translocation in the vicious cycle of malnutrition and enteric infections.

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PREVALENCE OF VIRULENCE GENES IN *CAMPYLOBACTER* SPP. ISOLATED FROM PERUVIAN CHILDREN UNDER TWO YEARS OF AGE

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Campylobacter jejuni and *C. coli* are major causes of gastroenteritis in children. Several virulence genes are associated in the process of infection. The aim of this study was to describe the prevalence of virulence genes in *Campylobacter spp.* isolated from Peruvian children under 2 years of age, with and without diarrhea. 100 *Campylobacter spp.* isolates were tested; 46% (59%, 27/46 *C. jejuni* and 41%, 19/46 *C. coli*) were from diarrhea, and 54% (56%, 30/54 *C. jejuni* and 44%, 24/54 *C. coli*) were from healthy control cases, from a cohort study in Lima, Peru. Standard procedures, and a multiplex PCR were performed to identify *Campylobacter* species. The following virulence genes were studied by PCR: *cadF*, *cdtA*, *cdtB*, *cdtC*, *cdt genes cluster*, and *iam.* For the *iam* gene three sets of primers were used. 99% of the *Campylobacter* strains analyzed presented *cadF,* only one *C. jejuni* isolated from a healthy control case did not present this gene. The *cdtA,* and *cdtC* genes were more frequently detected in *C. jejuni* than in *C. coli* (99% vs 17%) ($p<0.001$). *cdtB* was present in 76% of the strains, this gene was more frequently detected in *C. jejuni* than in *C. coli* (99% vs. 46%) ($p<0.001$). *cdtABC* gene was most frequently detected in *C. jejuni* 88% (50/57) vs. 5% (2/43) in *C. coli* ($p<0.001$). *iam* genes were more frequently detected in *C. coli* strains than in *C. jejuni* (94%, 88%, and 96% vs. 7%, 4% and 7%, for *iam1*, *iam2* and *iam3* sequences, respectively). There was no significative difference in the frequency of virulence genes between diarrhea and healthy control cases. *Campylobacter* spp. carrying relevant virulence genes could be isolated in both diarrhea, and in healthy control cases. Surveillance studies are important in endemic areas, in order to avoid epidemic outbreaks. Further studies are needed to look for additional virulence genes, and their expression, and also to determine if there is a relationship between the presence or absence of a specific gene, or associations of genes, and the clinical features of disease.

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WATCH AND WAIT: A VIABLE OPTION FOR CYSTIC ECHINOCOCCOSIS IN PREGNANCY

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The frequency of Cystic Echinococcosis (CE) in pregnancy is low, with approximately 1/20.000-30.000 new births in endemic areas. Consequently, experience in managing this condition during pregnancy is limited. In the current literature, some women are treated with surgery, others with percutaneous treatment or chemotherapy. We report our experience with six pregnant patients with CE seen in our center from 1990 to 2011. The mean age was 27 (range: 17-39). In all patients, CE was located exclusively in the liver. The cysts were all transitional and inactive (WHO IWGE standardized ultrasound classification); there were 3 CE3b, 2 CE4 and 1 CE5. We chose the "watch and wait" approach (expectant management) for each patient. All patients were monitored by ultrasound and serology and completed their pregnancies without significant complications between the 36th and the 40th week. Two patients delivered by caesarean section, one due to podalic presentation and the other as a cautionary measure against the risk of cyst rupture despite the fact that the cyst had remained unchanged during the course of pregnancy as happened with all the other women in the series. All newborns were healthy and have remained so to date. We conclude that the "watch and wait" approach for uncomplicated transitional and inactive CE of the liver is a viable option for pregnant women harboring liver cysts and because the cysts are not at risk of rupture during delivery, C-section can be avoided.

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HUMAN AND CANINE ECHINOCOCCOSIS INFECTION IN INFORMAL ABATTOIRS IN LIMA, PERU

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The complex cystic/canine echinococcosis has been described as a major public health problem among in livestock-raising regions of Mediterranean, Northern Africa, Central and Southeast Asia, New Zealand, South America (Peru, Argentina, Chile, Uruguay and southern of Brazil). In the life cycle of echinococcus granulosus, dogs play the role of definitive host (canine echinococcosis), whereas humans play the role of accidental intermediary host when they are infected by the accidental ingestion of eggs, developing cystic lesions after several years of the infection (cystic echinococcosis). This complex was previously reported in several studies performed in Peruvian rural areas and there are few reports of this problem in urban areas. Lima, an 8 million habitant's metropolis, has migratory patterns that have created regions in the periphery of this city where poor populations bring animals from endemic areas and slaughter them without veterinary supervision. A cross-sectional study was conducted in 8 informal abattoirs located in a peripheral district of Lima to assess the prevalence of cystic and canine echinococcosis among humans and dogs that live in these abattoirs. This study included abdominal ultrasound, serological and radiological evaluation of people who live in abattoirs and evaluation of dog samples by direct coproparasitoscopy and coproELISA; dogs that were positive according to coproELISA were evaluated by PCR and arecoline bromhydrate purge. Among 32 family members evaluated we found 2 (6.25%) subjects with liver echinococcal cysts. Eight of 22 dogs (36%) were positive to coproELISA, and four of 22 dogs (18%) were confirmed with E. granulosus tapeworms either by PCR or direct observation (purge). This study demonstrates the existence of authoctonous transmission of e. granulosus in urban Lima.

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ABSENCE OF MEGALOBLASTIC ANEMIA IN INDIVIDUALS INFECTED WITH *DYPHYLLOBOTHRIUM PACIFICUM*

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Human diphyllobothriasis is mostly caused by *Dyphyllobothrium latum* or *D. pacificum* and more rarely by other *Diphyllobothrium* species. It is acquired by ingesting poorly cooked fish containing infective larvae. The diagnosis is based on the identification of characteristic eggs in stools or morphology of proglottids, which are eliminated spontaneously with some frequency, and more recently helped by molecular techniques. *D. pacificum* (Nybellin 1931, Margolis 1956) is the only causal agent of diphyllobothriasis in the Western coasts of South America. Unlike in *D. latum*, infections, there are no reports of anemia or B12 vitamin deficiency caused by *D. pacificum*. We performed a systematic study in individuals diagnosed as carrying this tapeworm to assess its association with megaloblastic anemia and B12 vitamin deficiency. Between 2009 and 2011, 19 individuals diagnosed of diphyllobothriasis were invited to participate in the study and signe the approved consent form. Eleven (58%) were females, with ages ranging between 8 and 66 years, and 8 were male, with ages between 3 and 59 years. Patients had a 3 cc blood simple taken at baseline (not later tan 15 days after the diagnosis), processed for hematocrit, serum levels of B12 vitamin, and Wright smears for differential cell counting. A second simple was taken 90 days after treatment to assess changes in B12 levels after parasite expulsión. Fifteen participants accepted baseline blood collection, and 9 of them also had a second blood sample taken at day 90. All hematocrit values were in normal range. Both in males and females there were slight variations in white blood cell counts, with increased eosinophils in 8 (53%) cases at baseline, and 3 (43%) at day 90. Vitamin B12 levels were measured in 14 samples at baseline and 9 at day 90. All results were in normal range (243 -894 pg/ml), except for a patient with a baseline level of 199 pg/ml which dropped to 143 pg/ml 90 days after successful treatment. B12 levels were quite varied, with a median of 437 and interquartile range of 332 to 572.5 pg/ml. There is no association between *D. pacificum* infection and anemia, nor with vitamin B12 deficiency.

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ADHERENCE OF *TAENIA SAGINATA* ONCOSPHERE TO CHO K1 CELLS AND INTESTINAL CELLS

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The mechanism of *Taenia saginata* oncosphere adherence in the host has not been studied previously. To investigate the oncosphere adherence mechanisms will help to understand the parasite-host interaction, the immune system and develop an efficacy vaccine that could be preventing the adherence of parasite and consequently the host infection with the larval stage. The purpose of this study was standardized an *in vitro* model that helps to understand the adherence mechanisms of *T. saginata* oncosphere and the relationship with molecules involved in the parasite-host cell recognition process. We developed an *in vitro* adhesion model assay using a plate of 96 wells to evaluate the mechanisms of *T. saginata* oncosphere adherence. To determine the optimum number of *T. saginata* oncosphere to use in each assays, different amounts of biotinylated activated oncospheres (2500, 5000, 10000, 15000) were incubated on monolayer CHO-K1 (Chinese hamster ovary cells) or human intestinal monolayer cells: HCT-8 (Human colorectal adenocarcinoma cells), INT-407 (Caucasian intestine embryonic cells) and HT-29 (Human colorectal adenocarcinoma cells). To know the role of fibronectin in the oncosphere adherence, biotinylated-activated oncospheres were preincubated with different concentrations of fibronectin (FN) (10, 40, 80 and 100 ug/ml) and then incubate with the different monolayers cells lines that mention above, as a control was use oncospheres incubates with media alone. We found that the optimum number of biotinylated *T. saginata* oncosphere to use in each assays was 10000. And the oncosphere adherence to the cells (CHO, HCT-8, INT 407 and HT-29) was increase by fibronectin. But in the case of CHO cells the increase oncosphere adherence was dose dependent manner when was incubated with different fibronectin concentrations. We demonstrate that *in vitro* model using different cells lines are a useful model to study the adherence mechanisms of the parasite.

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CHILDHOOD NEUROCYSTICERCOSIS IN TROPICS: RECOGNIZING THE LINKAGE BETWEEN TRANSMISSION DYNAMICS AND AGRO ECONOMIC PRACTICES

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Childhood Neurocysticercosis (NCC) remains a serious neglected problem in marginalized communities in tropical countries due to lack of information and awareness, suitable diagnostic and appropriate prevention and control strategies. The manifestations are polymorphic; acute symptomatic seizures being the most common. Within this

background we conducted a hospital based descriptive study over the period of one year (July 2008 to June 2009) in the department of paediatrics at B.P Koirala Institute of Health Science (BPKIHS) a university teaching hospital in eastern Nepal. Our aim was to evaluate the patients with childhood Neurocysticercosis for their risk characterization and socio epidemiological profile. 32 children aged 1-14 yrs, presented with new-onset seizure and Neurocysticercosis during the study period. Neurocysticercosis was diagnosed on the basis of consistent Neuroimaging and/or diagnostic serum titer with compatible epidemiological background. 50% of the childhood NCC had partial seizure with secondary generalization, 25 % had primary generalized seizure and 25% had simple partial seizure. Seroprevalence of cysticercosis was found to be 75 %. CT scan head showed living cysts in 20% and calcified lesion in 6%.Kappa measurement of agreement between CT scan head showing live cysts and Ag-ELISA positive was found to be 76.3%. The most striking finding was linkage between evidence of Neurocysticercosis, transmission dynamics and Agro economic practices. Children from semiurban and rural pig farming community with unpenned animal farming, poor housing without safe drinking water were significantly associated a positive Ag-ELISA and neuroimaging evidence of Neurocysticercosis. Consumption of undercooked pork and raw vegetables and poor hand hygiene were additional risk factors. To conclude, Childhood neurocysticercos, has a strong linkage between its transmission dynamics and Agro economic practices related to livestock farming in tropics. There is a need to adopt concerted and focused strategies that put emphasis on health education, poverty alleviation as well as improved agro economic practices to prevent and eradicate this neglected disease from tropical countries.

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ESTIMATING THE NON-MONETARY BURDEN OF NEUROCYSTICERCOSIS IN MEXICO

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Neurocysticercosis (NCC) is a major public health problem in many developing countries where health education, sanitation, and meat inspection infrastructure are insufficient. Although NCC is endemic in many areas of the world and is associated with considerable socio-economic losses, very few studies have been conducted to evaluate the burden of NCC and there are no estimates from Mexico. This study provides the first estimate of disability adjusted life years (DALYs) associated with NCC in Mexico. DALYs lost for clinical cases of NCC in Mexico were estimated by incorporating morbidity and mortality due to NCC-associated epilepsy, and morbidity due to NCC-associated severe chronic headaches. Latin hypercube sampling methods were employed to sample the distributions of uncertain parameters and to estimate 95% credible regions (95% CRs). In Mexico, 460,519 and 305,319 individuals were estimated to suffer from NCC-associated epilepsy and NCC-associated severe chronic headaches, respectively. A total of 66,000 (95% CR: 29,600 - 124,400) DALYs were estimated to be lost due to these clinical manifestations, with 0.64 (95% CR: 0.29 - 1.2) DALY lost per thousand person-years of which 88% was due to NCC-associated epilepsy. The burden of NCC was comparable to the 2004 global burden estimates for other helminthic infections in Mexico, but lower than the NCC burden estimated in Cameroon. The numbers of DALYs associated with NCC are likely to be underestimated since only the clinical manifestations of epilepsy and severe chronic headaches were included. Even with this limitation, preliminary estimates suggest that healthy years of life are lost due to NCC in Mexico.

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PREVALENCE AND CO-OCCURRENCE OF *TAENIA SOLIUM* CYSTICERCOSIS WITH PORCINE GASTROINTESTINAL PARASITES IN CENTRAL TANZANIA: OPPORTUNITIES FOR INTEGRATED CONTROL MEASURES

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Taenia solium is a parasite transmitted primarily between humans and pigs leading into human neurocysticercosis, which results into disabilities, and hence, sufferings and economic losses. Pigs are the primary intermediate hosts harbouring parasite larvae leading into further economic losses due to pork condemnation. Because of its zoonotic nature, control of *T. solium* requires an integrated approach targeting both the human tapeworm carriers and cysticercotic pigs. Most of the previous *T. solium* control efforts have focused on inter-sectoral collaboration as one way of integrated approaches. However, the efforts have overlooked opportunities for inclusion of other important co-endemic parasites in control programmes. This is with exception of the current efforts to integrate *T. solium* control with that of schistosomiasis in some endemic regions. A cross-sectional study in Kongwa district of Dodoma region, central Tanzania established an overall prevalence of 14.9% (n = 309) of porcine cysticercosis based on lingual examination (Se ≈ 21%, Sp ≈ 100%); and *Ascaris* spp. (3.9%), *Trichuris* spp. (3.2%), Strongyle spp. (26.3%) and *Coccidia* oocysts (11.6%) based on coprological examination of 285 pigs. The overall worm burden ranged from 1700 - 2300 (median 700) egg per gram of faeces. Logistic regression analyses revealed significant clustering of porcine cysticercosis prevalence as well as that of GIT helminths by village (P = 0.001), though the village preference by the two infections was different. The GIT helminth infection was significantly higher in male than female pigs (P = 0.000) and in older than younger pigs (P = 0.010). The observed co-occurrence of porcine *T. solium* cysticercosis with porcine GIT helminth infections creates an opportunity for integrated control of *T. solium* in Tanzania. More studies are needed to explore other important co-infections in pigs and humans to fully utilize the opportunities for integrated control of *T. solium* in Tanzania.

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ASSOCIATION BETWEEN HIV INFECTION AND THE PROPORTION OF NCC LESIONS AMONG PATIENTS WITH NEUROLOGICAL DISORDERS IN THE EASTERN CAPE PROVINCE, SOUTH AFRICA

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There is little known about the effect of HIV on infection with *Taenia solium*-associated neurocysticercosis (NCC). The objective of this study was to estimate the cross-sectional association between HIV and NCC among patients with neurological disorders receiving care in two hospitals of Mthatha in the Eastern Cape Province (ECP), South Africa. Patients were consecutively sampled at routine visits to neurology and epilepsy clinics at

Nelson Mandela Academic Hospital (tertiary hospital) or through referral from Mthatha General Hospital (Level II hospital). Patients presenting with recent onset of epilepsy, seizures and severe chronic progressive headaches were eligible for inclusion in the study. As of April 12, 2011, 70 patients with neurological disorders were recruited. All patients received a full neurological examination and were offered a CT-scan of the brain if one had not been performed recently. All CT scans were evaluated by one of two radiologists for the presence of lesions suggestive of NCC. Among the 23 HIV positive and 33 HIV negative patients with available CT-scan results, 8 (36%) and 7 (22%), respectively, had lesions suggestive of NCC. This corresponds to a prevalence proportion ratio of 1.64 (95%CI: 0.69-3.89). Interestingly, 4 of the 8 NCC cases among HIV positive patients showed active lesions whereas there was only one such case out of the 7 NCC positive HIV negative patients.- More results are expected shortly which should increase the power to test for differences between these two groups.

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EVANS BLUE USE IN DELINEATION OF BLOOD BRAIN BARRIER DYSFUNCTION IN NEUROCYSTICERCOSIS IN SWINE

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Blood brain barrier (BBB) disruption has been suggested to play an important role in the inflammatory responses associated with neurocysticercosis (NCC). Experiments employing intravenous Evans Blue (EB) injection in *Taenia solium*-infected pigs to demonstrate presence, location and severity of BBB dysfunction in NCC in pigs are described. Untreated (U) naturally infected pigs or animals treated (T) with praziquantel 2-5 days earlier were studied. EB was injected 2h-2 days before euthanasia, the brains perfused with saline and/or formalin, gross evaluation of the brain performed and tissues collected for analyses. A protocol injecting EB 2 hr prior to sacrifice at 80 mg/Kg, 4 ml/kg of 2% EB in PBS appeared adequate and convenient. Praziquantel at 100 mg/ kg 48 hr earlier revealed larger and more intense blue pericystic regions in brains from T pigs. The number of blue cysts as opposed to clear, unstained ones in one U brain was 21/63 (25%) while in two T pigs blue cysts were 24/27 (89%) and 30/57 (53%). Preliminary results showed increased inflammatory scores in granulomas from treated pigs. Some cysts possessed one to several "blue dots" involving the cyst wall indicating localized sites of EB leakage; their frequency appeared to be increased in treated pigs (3.7% vs. 0.0%, 0.03%). Initial histological studies of cysts in situ showed these areas represent localized regions of host inflammatory response. Analyses of important expressed proteins by RT-PCR, semi-quantitative histopathogy of EB stained and unstained pericyst tissues are in progress. EB staining allows identification and histological and molecular characterization of lesions and blood vessels with BBB disruption. Inflammatory responses by the host against established cysts are initially localized to specific regions of the cyst. Correlation between enhancement seen in magnetic resonance imaging and EB staining in pigs is planned to elucidate the relationship between the histological changes observed and acute post-treatment edema that is characteristically associated with treatment of NCC.

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USE OF A NEW RT24 ELISA ASSAY IN DIAGNOSIS OF NEUROCYSTICERCOSIS

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Cysticercosis in humans and pigs is caused by infection with the cysticercus larval stage of the pork tapeworm *Taenia solium*. Neurocysticercosis refers to this infection in the brain and other nerve tissues; it is prevalent wherever pigs are allowed to roam for food and sanitation facilities are inadequate. Diagnosis relies on neuroimaging in conjunction with immunodiagnosis using the CDC enzyme-linked immunoelectro-transfer blot (EITB). The EITB detects antibodies to any of seven lentil lectin-bound glycoproteins (LLGP), which consist of a mixture of antigens that belong to three protein families of proteins, namely 50-kDa protein, and the 24/42-kDa and 8-kDa families. The most frequently recognized protein in the LLGP fraction is the 42-kDa protein, which is a homodimer of the 24 kDa protein. In this study we developed and evaluated an enzyme immunosorbent assay (ELISA) using recombinant T24 (rT24) for laboratory identification of cysticercosis. The use of peroxidase conjugated Protein G as the secondary antibody allows detection of T24 specific antibodies from various species, including both human and porcine immunoglobulins. We analyzed a panel of 412 sera composed of 104 sera from patients with neurocysticercosis, 113 sera from persons with other parasitic infections, and 195 sera from individuals who reside in non-cysticercosis endemic areas and from non-travelers residing in the US. The optimized assay has a sensitivity of 95.2% in patients with 2 or more cysts and a specificity of 94.5%. With the development of this recombinant protein based ELISA method, reliable diagnostic tests for neurocysticercosis can be made more widely available. In addition, this method is suitable for serosurveillance in both human and porcine populations in cysticercosis control programs.

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EVALUATION OF THE IMPACT OF INFORMATION TRANSMISSION FROM CHILDREN TO PARENTS IN A PROGRAM TO CONTROL CYSTICERCOSIS IN THE NORTHERN COAST OF PERU

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The access to information is a cornerstone for regional development strategies as well as for control programs; nevertheless the great challenge for the use of these strategies in rural areas is the adequately spread of information among community members; one technique, that has been used to face this problem, is the "reverse intergenerational transmission". The complex taeniasis/cysticercosis is highly endemic in rural areas of the northern of Peru, and previous studies have demonstrated that an adequate information about diagnosis of taeniasis could reduce the burden of taeniasis and so on the risk to develop cysticercosis among susceptible community members. We performed a community randomized trial in 11 villages to explore the changes in the demand proportion of parasitological tests between areas where children received information at school about taeniasis/cysticercosis (treatment group, 3 villages, n=2220) and areas where this information was provided in a general way to the entire population (control group, 8 villages, n=2604). In both groups we performed a baseline and a post treatment evaluation of demand proportion. Baseline evaluation demonstrate a higher demand proportion in control group (51% vs 33%, p<0,05); nevertheless this difference in

proportions was reduced after the intervention, and being the almost the same we found in our treatment group (35% vs 31%, p=0.0036). In addition we used each group as their own control, and we found that among treatment group there was not any statistical difference between the demand proportion before and after the intervention (33% vs 31%, p= 0.1125) whereas there was an statistical significant reduction among control group (51% to 35%, p<0.05). Study's findings will contribute to a better understanding of the role that rural schools can play in the distribution of information relevant to the development of their communities, and also give us greater insight about relationships between parents and children within household and its effect on decision-making and family safety.

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SEROPREVALENCE OF ANTIBODIES AGAINST *TAENIA SOLIUM* CYSTICERCOSIS AMONG U.S.-BOUND REFUGEES

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Neurocysticercosis (NCC) is a disease caused by central nervous system infection by the larval stage of the pork tapeworm, *Taenia solium*. It is a leading cause of adult-onset epilepsy in developing nations. There are increasing case reports of NCC among refugees resettled to the United States and other nations, but the underlying prevalence among refugee groups is unknown. We tested stored sera from the Centers for Disease Control and Prevention Migrant Serum Bank for antibodies against *T. solium* cysticercosis using the enzyme-linked immunoelectrotransfer blot (EITB LLGP). Sera were selected from refugee populations in which published reports suggested *T. solium* endemicity in the countries of origin. Our final sample included a total of 2001 sera from resettled refugees from Laos (Hmong), Burma, Bhutan and Burundi. The crude seroprevalence was high among all four populations tested, including refugees from Burma (23.2%, 95% CI 19.5-27.0%), Laos (18.3%, 95% CI 14.9-21.7%), Bhutan (22.8%, 95% CI 19.1-26.5%) and Burundi (25.8%, 95% CI 22.0-29.6). The aggregate seroprevalence was statistically homogenous across categories of age and gender. Within individual refugee groups, statistically significant differences in the odds of exposure were noted with respect to age, gender, camp of origin and country of birth. The seroprevalence in all four groups was comparable to the seroprevalence in well-characterized endemic communities in Latin America where there is substantial morbidity related to NCC. Clinicians attending refugee populations should consider NCC in patients with seizure, chronic headache or unexplained neurologic manifestations. Improved understanding of the prevalence of epilepsy and other diseases associated with NCC could guide recommendations regarding evaluation and treatment of refugees before, during and after resettlement.

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EMERGING ZOONOTIC DISEASES OF COMPANION ANIMALS IN NAMIBIA

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Companion animals are increasingly implicated in the transmission of zoonotic diseases of veterinary and public health importance. Yet, in many developing Sub-Saharan countries, the impact of such zoonotics is overlooked despite their possible effect on large populations of immune-compromised persons (HIV). Such is the case in Namibia, where a high prevalence of HIV and tuberculosis together with goal of malaria elimination limit resources available to address the potential risk of zoonotic pathogens. The aim of the study is to identify zoonotics, known to infect humans and their companion animals, which may be important in the disease epidemiology of Central Namibia. The setting is the Rhino Park Veterinary Clinic located in central Windhoek. Some pathogens identified include: 1) *Ehrlichia canis*, known to infect dogs and humans, was identified by microscopy and ELISA (ImmunoComb, Biogal Laboratories). Recently, 18 (43%) of 42 suspected cases were positive by microscopy. Another study found 59.2% of 76 pet dogs tested were medium or strongly reactive positive for *E. canis* antibodies. Of 30 stray dogs tested, 13 (43.3%) were reactive. 2) *Mycobacterium tuberculosis* was identified from 5 granulomas taken from the ears of 5 dogs from the low-income neighborhood. This is significant as Namibia has the fourth highest TB incidence rate in Africa. 3) *Dirofilaria repens* was also identified microscopically in 10 suspected dogs, indicating possible importance for dogs and humans. 4) *Anatrichosoma sp.*, a rare nematode only reported once in Africa and known to infect humans, was recently identified from the footpads of a cat. Other studies are currently evaluating the prevalence of *Rickettsia*, *Babesia*, *Hepatozoon*, *Bartonella* and the possibility of *Leishmania* in dogs from central Namibia. Together, these studies are identifying possible emerging zoonotic pathogens which could pose veterinary and public health risks through companion animals in Central Namibia but are not considered important enough to monitor by the national health system.

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DEVELOPMENT OF AN EFFICIENT NON-HUMAN PRIMATE SPOROZOITE CHALLENGE WITH *PLASMODIUM KNOWLESI* VIA *ANOPHELES DIRUS (SENSU STRICTO)* MOSQUITO BITES

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Plasmodium knowlesi is a virulent infection in both non-human primates and humans. Thus, *P. knowlesi* infection of NHP offers a potentially useful vaccine model. In previous challenges of rhesus macaques with *P. knowlesi*, we have utilized intravenous injection of sporozoites isolated from the salivary glands of hand-dissected *Anopheles dirus* mosquitoes that had been infected via feeding on gametocytemic donor monkeys. The direct introduction of a relatively large number of sporozoites into the blood stream may inadequately assess the efficacy of candidate malaria vaccines by bypassing the interaction of the sporozoites and the immune system during transmission by the mosquito. This concern applies particularly to malaria vaccines designed to induce antibody responses that neutralize the sporozoite following deposition into the skin. In this study, we detail the experiments which led to the establishment of procedures required for a successful mosquito bite challenge with *P. knowlesi* using the rhesus monkey model.

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SEROLOGIC SURVEILLANCE OF RICKETTSIAL DISEASES IN NORTHEASTERN CAMBODIA

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Rickettsial infections have been report worldwide but only limited studies have been performed in Cambodia. Rickettsioses are arthropod-borne diseases caused by intracellular bacteria of the genera Rickettsia and Orientia and are classified into 3 groups: spotted fever group (SFG), typhus group (TG) and scrub typhus group (STG). Specimens from patients enrolled in a fever syndromic surveillance study from northeastern Cambodia near the Lao PDR border enrolled between July-December 2010 were tested for the presence of specific antibodies against SFG, TG, and STG rickettsiae by enzyme linked immunosorbent assays (ELISA) developed at the Naval Medical Center (Silver Spring, MD). Convalescent sera positive for antibodies specific to one of the three rickettsial groups were detected in 45 of 188 (23.9%) samples and were individually positive in 30 (16.0%), 6 (3.2%) and 9 (4.8%) cases for SFG, TG and STG, respectively. Titrations were performed on positive convalescent specimens and their corresponding acute specimens. Recent infections defined by four fold rising antibodies titers or seroconversion were determined in one case of SFG, one case of TG and three cases of STG rickettsioses. These results suggest that rickettsial infections may be a common etiology of fever for this area. Continued monitoring of this population and molecular characterization of these specimens will provide further insight to the epidemiology of rickettsioses in this area.

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PREVALENCE OF ACTIVE CONVULSIVE EPILEPSY (ACE) - DATA FROM INDEPTH EPILEPSY STUDIES

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Epilepsy is a common neurological disorder affecting nearly 70 million people worldwide, majority of who live in Low and Middle Income Countries (LAMIC). Although these estimates are global, they are based on very sparse data from LAMIC. Additionally, there is considerable heterogeneity of estimates from LAMIC and it's not clear if this is methodological. In the current Epilepsy research programme, we are conducting studies to determine prevalence, risk factors and mortality using standardized tools and methods in 5 Health and Demographic Surveillance Sites (HDSS) in sub-Saharan Africa (SSA) under the INDEPTH Network (http://www.indepth-network.org). These include Kilifi in Kenya, Agincourt in South Africa, Ifakara in Tanzania, Iganga/Mayuge in Uganda and Kintampo in Ghana. At the ASTMH meeting, I plan to present data on prevalence of ACE in four of the five sites where the surveys are already complete. Three-stage surveys were used to identify cases of ACE in Agincourt, Ifakara, Kilifi and Iganga-Mayuge HDSSs. Diagnosis was made by clinicians through clinical history and neurological examination. Prevalence was estimated as the proportion of identified cases in each site. We used multiple imputation (MI) to adjust for attrition between survey stages. Forest plots were used to assess heterogeneity and a random-effects model was used to derive pooled prevalence. The crude prevalence ranged between 2.3/1000 (95% CI: 2.0-2.6) in Agincourt to 3.9/1000 (3.5-4.3) in Ifakara. When adjusted for loss-to-follow-up between stages, prevalence ranged from 2.7/1000 (2.3-3.0) in Agincourt to 7.1/1000 (6.5-7.8) in Ifakara. There was statistically significant heterogeneity of ACE across the sites. Accounting for between-site variation, the pooled median prevalence was 4.5/1000 (3.0-6.7). In conclusion, this is the largest study of prevalence of epilepsy in LAMIC. We focused specifically on ACE because of its important implications for stigma, disability and mortality and it's also most amenable to therapeutic intervention. Despite using similar methodology and definitions of epilepsy, we found significant heterogeneity across SSA suggesting possible differences in types and distribution of risk factors.

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TIMELINESS OF CHILD VACCINATIONS IN KAMPALA, UGANDA

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Most studies that report on child utilization services report on vaccination coverage. Timely vaccination is important to ensure optimal response to vaccines and to have early disease protection yet data on timeliness of child vaccines is scanty. We examined delay in receiving recommended vaccines and the factors influencing this delay among respondents with children under 2 years in Kampala. This household survey used cluster sampling methods with a sample of 825 respondents. Study participants responded to a questionnaire with the following components: attitudinal factors like perceived benefits from vaccinations, social factors such as support from important others like spouses and self-efficacy factors like being able to cope with poor communication with the spouse. Mobile telephones were used to collect data. Multinomial logistic regression was used to identify influences on delayed vaccination for each dose. Delayed vaccination was considered for each dose: DPT-Hib-HEB1 (>2 months), DPT-Hib-HEB3 (>6 months), and measles (>12 months). Vaccination was delayed for DPT-Hib-HEB1 in 25.5% of the children with a median delay of 49 days, in 15.4% of children for DPT-Hib-HEB3 with a median delay of 121 days, in 24.6% of children for measles with a median delay of 413 days. Delayed vaccination for DPT-Hib-HEB1 was reduced by fathers involvement in decision making for childhood vaccination (OR=0.69, 95% CI= 0.48-0.99) and if respondents reported that they had less work (OR= 0.64, 95% CI= 0.40-0.98). Those that said they found it difficult to discuss vaccination issues with their partners tended to be delayed for both DPT-Hib-HEB3 (OR= 0.30, 95% CI= 0.12-0.82) and measles (OR= 0.59, 95% CI= 0.37-0.94). For measles, respondents that said the father had visited the vaccination post were less delayed than those that did not report this (OR= 0.51, 95% CI= 0.28-0.90), and respondents that said they would not immunize a child with fever were more delayed than those that said they would immunize a child with fever (OR= 1.64, 95% CI= 1.04-2.57). Vaccination programs should increase male involvement to improve timeliness of child vaccinations.

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COMPARISON OF MENTAL HEALTH STATUS AMONG ADULT REFUGEE AND NON-REFUGEE POPULATIONS IN ORU-IJEBU, SOUTHWESTERN NIGERIA

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This study aimed to assess and compare the mental health status of adult refugees and non-refugees in Oru-Ijebu, South-Western Nigeria. The prevalence of mental health problems among refugees is generally known to be relatively high. There is a dearth of studies on the mental health status of West African refugees. A community-based comparative cross-sectional study of refugees and non-refugees was done. Respondents were chosen by a random cluster sampling technique. Data was collected using Mini-International Neuropsychiatric Interview (MINI) in an interviewer-administered questionnaire. Predictors of poor mental health was determined using logistic regression. Level of statistical significance was set at 5%. A total of 444 refugees (45.7%) and 527 non-refugees (54.3%) were interviewed. The mean age of respondents was 34.8±12.8years among refugees and 33.3 ±8.1years among non-refugees($p<0.05$); about 60% were males in both groups. About 65%

of the refugees were Liberians while 99% of the non-refugees were Nigerians (p<0.001). Poor mental health was reported by 61.9% of refugees and 34.7% of non refugees, p<0.001. Prevalent mental health problems among refugees and non-refugees included depression (45.3% vs. 19.4%, p<0.001), Post-Traumatic Stress Disorder (34% vs. 13.7%, p0.05), alcohol abuse (13.5% vs.19%, p<0.05) and suicide ideation (11% vs. 9.5%, p>0.05). The predictors of poor mental health were refugee status (OR: 4.3; 95%CI: 2.194 - 8.431), poor quality of life (OR: 2.068; 95%CI: 1.601 - 2.672), current medical problems (OR: 2.9; 95%CI: 2.105 - 3.994) and poor housing (OR: 1.546; 95%CI: 1.178 - 2.209). The mental health of refugees was poorer and almost all the mental health problems identified were more prevalent among the refugees. There is an urgent need to improve housing, medical care and employment opportunities in the Nigerian refugee camp. An integrated system of support for those with mental health problems will also go a long way to improving mental health at the community level.

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ADHERENCE TO COMPLEMENTARY FEEDING RECOMMENDATIONS FOLLOWING AN INTERVENTION FOR REDUCTION OF CHILDHOOD MALNUTRITION IN KENYA

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Globally, the issue of childhood malnutrition contributes to more than 10% of the world's disease burden. In Kenya, malnutrition rates have risen for the past two decades, further exacerbating the challenges of poverty. Adequate nutrition during the "critical period" from 6 months-2 years of age is essential to the reduction of malnutrition rates as well as many of the long-term, irreversible sequelae that result from malnutrition during this period. In order to address malnutrition, various interventions have been implemented - many focusing on maternal and early childhood complementary feeding practices, appropriate breastfeeding practices, and proper hygiene and sanitation measures. The most effective interventions are able to combine nutrition education and provision of nutrient-rich foods. Evaluation of the sustainability of these early interventions, as well as long term outcomes are necessary to adequately assess the effectiveness of the intervention and gain insight for future interventions. Most importantly, outcomes need to be assessed not only based on an anthropometric basis to measure rates of malnutrition, but also other long-term outcomes of the education and nutritional provision; this includes the continuation of improved nutritive content of foods given to the children after an intervention consisting of education and food distribution has ended. In this study, we will evaluate the adherence to complementary feeding recommendations nine months after the cessation of supplemental food provisions. We will evaluate the nutritional content of the children's current diet and foods given to the children following the cessation of the study in order to assess the sustainability and adherence to improved nutritional supplementation after the intervention. We will also explore the correlation between specific nutrients and food groups (e.g. animal-source foods) and child growth and malnutrition prevalence.

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GLOBALIZATION AND INDIGENOUS HEALTH: A CASE STUDY OF THE SOCIAL AND ENVIRONMENTAL CONTEXT OF ACHUAR CHILD HEALTH IN AN OIL EXTRACTION ZONE IN THE PERUVIAN AMAZON

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Indigenous peoples internationally have poorer health status than non-indigenous populations. In the Peruvian Amazon, remote indigenous populations face dual challenges of isolation from health services and increasing exposure to rapid social and environmental change caused by recent expansion of resource extraction activities in their territories. This research uses interdisciplinary methods to explore social and environmental determinants of health for children aged <5yrs in a case study of indigenous Achuar communities in the Corrientes river basin. Malaria and diarrhoeal diseases are endemic in the region. The communities' living, fishing and hunting territories straddle two internationally run oil concessions and oil extraction activities contribute to community members' daily environments. Indigenous Amazonian populations have distinct health belief systems based on their relationship with their physical and social environment. Aiming to respect and learn from local conceptualisation of health and illness, this research addresses determinants of young children's health from multiple perspectives. Firstly, qualitative methods were used to explore community members' (n=30) and local stakeholders' (n=30) perspectives of factors influencing young children's health. Secondly, quantitative social epidemiological methods were used to characterise structural (e.g. ethnicity) and proximal (e.g. material circumstances) determinants of health based on the framework proposed by the WHO Commission on Social Determinants of Health (n=138 children aged <5 yrs in 97 households). Qualitative results found that respondents across groups identified food - access to sufficient and appropriate kinds of food - as a key child health determinant. 96% of households receive donated food. Also highlighted were; the biological and spiritual importance of the river, protection from mosquitoes, and access to economic resources. Quantitative analysis compared socio-economic and biomedical child health indicators in the case study setting with other indigenous and non-indigenous poor rural Peruvian communities outside oil extraction zones. Research findings have implications for policy development to protect children's health in indigenous communities increasingly exposed to large scale extraction activities across the Peruvian Amazon.

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KNOWLEDGE AND ATTITUDES OF MEDICAL PERSONNEL IN TRANSFUSION MEDICINE IN BAMAKO, MALI

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The objective was to study the knowledge and practice of medical personal in transfusion medicine in the district of Bamako and Kati. The study was conducted in different health centers in the district of Bamako. Physicians' knowledge and practice were assessed using a questionnaire. The study population consisted of medical specialists (15%), general practitioners (21.4%), nurses (41.6%), and midwives (22%). Sixty-six point nine percent of the population had not received training on blood transfusion since they graduated. The general knowledge about blood transfusion was not well in 37.6% and unknown in 30.3%. Knowledge of blood products, their traits, and possible accidents related to their use were not adequately controlled. Knowledge of stages of the blood transfusion was good in 78.6% of respondents. The practice and conduct in case of accidents was good in 42.9% of the time. It was clear from our study that there was no real link between the training received, the level of knowledge and quality of transfusion practice, and the conduct in case of accidents or incidents in Bamako and Kati. Neither training nor age had any influence on the level of knowledge and practice of transfusion. There is a real and immediate need to educate clinicians on blood use as well as to develop guidelines on blood transfusion, monitoring and evaluation. Educational materials concerning transfusion medicine practice and use of blood products should be provided for practicing physicians working in these hospitals. Compliance with the indication for blood transfusion is the first step of blood safety. Continuous medical education programs should be offered in order to improve the level of transfusion medicine in Bamako, Mali.

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"*IMAGINE ONE DAY IT'S YOU*": EMPLOYMENT EXPECTATIONS VIS-À-VIS PRACTICE COMPETENCIES BASED ON A "DREAM JOBS" ASSIGNMENT IN AN MPH GLOBAL HEALTH PRACTICE COURSE

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Global health is an emerging specialty within schools of public health and medicine as well as new interdisciplinary graduate programs and campus-wide institutes. Recent attempts have sought to define the specialty and develop practice competencies. Created in 2004, the Global Health Department at the University of South Florida's College of Public Health offers MPH, MSPH, DrPH and PhD degrees with 198 students in four concentrations: global communicable diseases, global health practice (GHP), disaster management/humanitarian assistance, and community-oriented tropical health. For a required MPH course in the GHP track, a "dream jobs" assignment was devised to assess student aspirations. Three cohorts undertook an internet search for five desirable positions upon matriculation. The task was to summarize employer type, posting locale, job title, job specialty, primary duties, competencies, skills/qualifications and salary. The sample was 69 students and 345 job descriptions. In this paper, we present results for employer type, job title, job specialty and salary. We found that 63% of desired jobs are with multilaterals + NGOs and 24% with governmental + bilaterals. There was a marked preference to reside in a resource-rich country. Almost two-thirds (63.2%) of the job titles can be categorized as program manager/ project director (31.6%), public health advisor/coordinator (16.3%), or researcher/ epidemiologist (15.3%). The top-ranking topical specialties were program planning/ policy/ administration (45.9%) and infectious diseases (13.3%). Expected salary ranged from ~US$30k-150k, with a mean of ~77k. The cohorts exhibited interesting differences given the brief 3-year period of this exercise. In particular, M&E has become more prevalent. We discuss how these findings may relate to core values and competencies, and how such surveys might better inform decisions regarding degree requirements, curricula, and competencies as well as recruitment and placement strategies.

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EVALUATING IMMUNIZATION RATES AND BARRIERS TO IMMUNIZATION FOR CHILDREN 1 TO 5 YEARS OLD IN RURAL INDIA

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The Jamkhed villages of Maharashtra, India, have maintained high vaccination rates for over two decades under the care of Comprehensive Rural Health Project (CRHP). In 2004, the Indian government took over the responsibility for vaccination. This study assesses vaccination coverage and barriers to vaccination for children since the handover of responsibility. A cross-sectional study of vaccination coverage for the six WHO recommended vaccines necessary before age 1 was performed using an in-person, household survey of five villages outside of Jamkhed during August, 2010. Mothers from 190 households with 242 children aged 1-5 were surveyed. Subsequently, 11 focus group discussions were held to evaluate mothers' overall knowledge of vaccination, and barriers they perceived to effective immunization. Results were coded and analyzed for common themes. Of the 242 children assessed, 100.0% received all 3 doses of the Oral Polio Vaccine, 99.2% received all 3 doses of DPT and Measles vaccine, and 98.8% received the BCG vaccine. The most common barriers to immunization were poor communication of the immunization distribution schedule, parents' concerns over missed wages/ workdays when accompanying their children for vaccination, as well as, various challenges resulting from nomadic lifestyle. This study suggests that vaccination coverage for children under 1 year continues to remain high even after a transfer of responsibility to the Indian government. The identified barriers to effective immunization for the children should be addressed by better outreach, increasing number of health workers, and effective scheduling of vaccination.

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TREND IN STIGMA AND DISCRIMINATION TOWARDS PEOPLE LIVING WITH HIV/AIDS IN ADDIS ABABA, ETHIOPIA; A QUALITATIVE APPROACH

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HIV prevalence rate in Ethiopia is 2.1%. Stigma toward people living with HIV/AIDS (PLWH) contributes to reluctance to seek care, and hampers provision of adequate care. We attempt to study the extent and trends of stigma and discrimination, and determine causes and possible solutions. In urban Addis Ababa, in 2009, we conducted twelve focus groups, including five groups with HIV+ subjects and seven groups of subjects with unknown status or HIV- (total n=43). Each focus group interview included 3-4 persons and lasted 90 minutes. Community perception and attitudes towards PLWH, reasons for stigmatization, trends in levels of discrimination, and possible solutions were evaluated. We transcribed, coded, analyzed, and presented data based on HIV status, education, and interaction with the HIV community. Major themes, concepts and recommendations were developed. The majority of all subjects stated that stigma has generally improved in recent years. More HIV+ subjects believe discrimination is still a significant issue, while more non-HIV+ subjects believe most people in the community no longer discriminate. The predominant reason for discrimination is general lack of awareness about the disease and virus. Level of education, prior experience with HIV+ community, and knowledge of general effectiveness of medical treatment were important contributing factors. Discrimination and stigma towards PLWH still exist as significant barriers to medical care. Improved awareness through educational programs and media, addressing social factors such as education and development, and enhanced availability of effective treatment could help reduce stigma and discrimination, and these measures need to be reinforced.

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RAPID ASSESSMENT OF CHOLERA MORTALITY, ARTIBONITE, HAITI, 2010

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During the first month of Haiti's cholera outbreak, which began in October 2010 in Artibonite and Centre Departments, the case fatality rate exceeded 6%. Cholera-naïve populations often lack knowledge critical to prevent death. We evaluated cholera mortality in Artibonite to understand care-seeking behavior and treatment of patients who died. We defined a cholera decedent as a person ≥5 years old who died from acute, watery diarrhea, with illness onset after October 16, 2010. Decedents were identified first from health facilities and subsequently through interviews with decedents' families and neighbors. We obtained information on demographics, illness severity, health-seeking behaviors, treatment, and cholera awareness. We identified 87 cholera decedents, of whom 48 (55%) died in a health facility and 39 (45%) in the community; 33% of deaths occurred in the first week of the epidemic. Median age

was 50 years (range, 5-100 years); 33% were female. Median time from illness onset to death was 20 hours (range 3 hours to 7 days) for health facility decedents and 12 hours (range 2 hours to 8 days) for community decedents. Of 48 health facility deaths, 26 (54%) occurred after overnight admission. Among 39 community decedents, 23 (59%) did not seek medical care. Barriers to care-seeking included: not suspecting the illness was cholera (69%) and distance to health facility (26%). Of community decedents, 30 (77%) did not receive treatment with oral rehydration salts (ORS) at home. Observations of 87 decedents' households found 57 (65%) with no ORS sachets available. Findings from this assessment suggested that early in the 2010 Haitian cholera epidemic, death occurred rapidly. For many decedents, care was either inadequate or non-existent. Cholera mortality in Haiti can be reduced through ORS availability in the community, promptly seeking care, and assuring that health care workers are trained in appropriate disease management.

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RODENT RECOLONIZATION RATES AFTER INTENSIVE TRAPPING IN RURAL VILLAGES OF SIERRA LEONE

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Rodent trapping in homes and villages is often considered as a control measure for rodent-borne diseases such as Lassa fever and hantavirus pulmonary syndrome. However, the long-term feasibility of this approach is highly contingent on the rate of recolonization. We conducted a pilot study aimed at assessing the rate of rodent recolonization after intensive trapping. Three rural villages (Jorpowahun, Yawei, and Bumpeh) in eastern Sierra Leone, an area endemic for Lassa fever, were selected based on similar size (between 30-60 structures), type of house construction, and economic background. Rodent trapping was conducted on consecutive nights in each village using Sherman live-traps until trap success fell below 2% (considered "trap-out"). Rodent species was identified via morphometric analysis before sacrificing animals and obtaining organ specimens as well as rodent carcasses for future testing. The trapping process was repeated at four (Jorpowahun), six (Yawei) and eight (Bumpeh) weeks. The mean time to initial trap-out in the three villages was 13.7 (range 7-20) days. The time to trap-out on follow-up visits was 8.0 (4 week village), 7.0 (6 week village), and 4.0 (8 week village) days, with a mean of 6.3 for all three villages, or a mean reduction of 7.3 days (54%) compared with the initial trap-out time. The mean reduction in the number of rodents trapped for all three villages was 75.8%, indicating an observable effect 2 months after intensive trapping. *Mastomys* and *Rattus* species were the primary rodents trapped at both sessions at all villages. However, the first trap session showed greater numbers of other small mammal genera, including *Crocidura*, *Hylomyscus*, *Praomys*, and *Mus* species. We plan to expand these preliminary studies to further time points to assess the impact of intensive rodent trapping on longer-term rodent population density and species diversity, and ultimately the impact on human infection with rodent borne pathogens.

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INFECTIONS ASSOCIATED WITH SEVERE MALNUTRITION AMONG OUTPATIENT CHILDREN IN BAMAKO, MALI

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Malnutrition is a contributing cause in more than half of children's deaths. Severe malnutrition(SM) predisposes affected children to various infections. There are few epidemiologic data on invasive bacterial infections among malnourished children in sub-Saharan Africa. To better define infections associated with SM among children in developing countries, we conducted a study on the infections associated with SM in outpatients < 36 months of age at the Emergency Department (ED) of Hôpital Gabriel Touré in Bamako. Children with fever ≥39°C or syndromes compatible with invasive bacterial disease were eligible; blood and relevant body fluid were cultured. Bacteria were identified by standard microbiologic techniques. We calculed anthropometric measurements and transformed into percentiles and z-scores with the software calculates WHO Anthro (Anthropometric calculator) version 3.1,2010. Nutritional status was evaluated by WHO Child Growth Standards. Poor nutritional status was defined as -3≤ z-score <-2 for moderate malnutrition and z-score <-3 for severe malnutrition. A total of 4877 outpatients between January 2008 and December 2009 were included, among them 1364(27,9%) had a poor nutritional status, among the malnourished 646(13.2%) had SM. Among the severely malnourished diarrhea was the most frequent diagnosis 208 (32,1% p<0.01) followed by pneumonia 187 (28.9% p<0,01) and bacteremia 87 (13,4% p<0.001). Among those with severe malnutrition and bacteremia, *Streptococcus pneumoniae* was the most commonly isolated pathogen 46 (54.6% p= 0.01) followed by nontyphoidal salmonella species 22 (25,2% p= 0.05). In conclusion, SM is associated with diarrhea, pneumonia and bacteremia in Malian children. Streptococcus pneumonia and nontyphoidal salmonella are the most commonly isolated pathogen in malnourished children.

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THE SEROPREVALENCE OF HEPATITIS B SURFACE ANTIGEN IN IMMIGRANTS AND REFUGEES: SYSTEMATIC REVIEW AND META-ANALYSIS

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Hepatitis B virus (HBV) is an important global health problem that infects 350 million people worldwide and leads to 1 million premature deaths annually. In the last four decades, low hepatitis B endemic countries have received an increasing number of immigrants and refugees from HBV-endemic countries, but in many host countries they are not routinely screened for this infection. We conducted a systematic review of the seroprevalence of chronic HBV infection in the immigrant and refugee population arriving in low hepatitis B endemic countries to estimate the burden of HBV in this population. Four electronic databases (Medline, Medline In-Process, EMBASE and Cochrane Database of Systematic Reviews) were searched from January 1950 to February 2011 to identify published studies in English, French and Italian, reporting the seroprevalence of hepatitis B surface antigen (HBsAg) in the immigrant and refugee population. The data were pooled (overall, and stratified by region of origin and immigrant class) using a fixed-effect meta-analysis and heterogeneity was assessed using the I-squared statistic. A total of 824 studies were identified, 149 full text articles were evaluated, and 96

articles were included, representing 167,562 immigrants and refugees. The overall pooled seroprevalence was 4.3% (95% CI: 4.2% - 4.4%). The HBV seroprevalence differed significantly by region of origin; East Asia and Pacific [seroprevalence 10.8% (95% CI: 10.5% - 11.0%)], Sub-Saharan Africa [9.8% (8.4-11.2%)], Eastern Europe/Central Asia [2.8% (2.6-3.0%)], South Asia [1.5% (1.2-1.8%)], Middle East and North Africa [1.4% (1.2-1.6%)], and Latin America/Caribbean [1.0% (0.9-1.1%)]. The pooled overall seroprevalence of HBV in refugees [5.1% (95% CI: 5.0-5.2%)] was higher than that found in immigrants [2.7% (2.6-2.9%)]. Seroprevalence of HBV in immigrants and refugees reflect rates in their countries of origin. Those from East Asia and the Pacific, Sub-Saharan Africa and Eastern Europe/Central Asia have the highest seroprevalence of HBV and should be considered for screening for this infection.

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THE IMPACT OF MATERNAL ANTENATAL TETANUS VACCINATION ON THE IMMUNOGENICITY OF THE CONJUGATED TETANUS-*HAEMOPHILUS INFLUENZA* TYPE B VACCINE IN INFANTS IN COASTAL KENYA

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Antenatal vaccination of tetanus has reduced the rates of neonatal tetanus due to the transplacental transmission of neutralizing anti-tetanus toxin antibodies. Often women are immunized repeatedly with tetanus toxoid (TT) during a pregnancy and over multiple pregnancies. Because TT antibodies can cross the placenta, it is possible that antenatal immunization may induce immune tolerance or deviation in the fetus that could impair response to the TT-*Haemophilus influenzae* Type B (Hib) conjugate vaccine during infancy and early childhood. To examine this possibility, we correlated the frequency and timing of TT immunization during pregnancy with TT and anti-PRP (Hib) specific IgG responses in infants aged 6 to 36 months in Coast Province, Kenya. Mothers were recruited at the antenatal clinic at Msambweni District Hospital on the south coast of Kenya from June 2006-May of 2009. Maternal tetanus vaccination was performed during antenatal visits and documented. Mothers received 0-5 vaccinations, depending upon parity. Infants were vaccinated with the pentavalent vaccine (DTP-Hib,HepB) at 6, 10, and 14 weeks. Blood samples were collected at six month intervals from birth to age 3 years in children enrolled in the study. Infant response to TT and Hib vaccinations were measured by ELISA. A total of 248 mother-child pairs were analyzed. Mothers received a mean of 1.6 doses of TT vaccine during pregnancy (Range = 0 to 5). The data trended towards a negative correlation between number of maternal TT vaccinations received and infant antibody response to TT vaccination, but was not statistically significant (r= -0.12, p= 0.16). Infant antibody response to Hib vaccination at all time points was not significantly correlated with number of maternal tetanus boosters received. Timing of TT vaccination (1st, 2nd, or 3rd trimester) was not significantly correlated with infant titers. The percentage of infants with protective levels of antibodies against tetanus and Hib at 12 months were >90% and >98%, respectively. Multiple antenatal tetanus vaccinations did not diminish the immunogenicity of the TT-Hib conjugated infant vaccination in our Kenyan cohort. The vast majority of infants had protective levels of both TT and Hib antibodies at 12 months. These findings support the continued use of the pentavalent vaccine in infants, and aggressive maternal TT vaccination to prevent the morbidity and mortality of neonatal tetanus in the developing world.

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MOBILE PHONES AS DISRUPTIVE AGENTS IN THE PATHWAY TO MORTALITY DURING EMERGENCY OBSTETRIC CRISES IN RURAL BANGLADESH

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Over the past decade, rapid expansion of cellular networks in resource-poor settings and decreasing costs of mobile phone ownership have raised this technology's potential as a public health tool. We explored phone ownership and use by pregnant women in a large, rural population of South Asia, using data from the ~500km2 JiVitA study area, one of the largest population research sites in the Gangetic region. Since 2001, we enrolled and followed > 100,000 pregnant women into large randomized controlled nutrition trials to improve materno-fetal-infant health and survival. Measuring access to and use of mobile phones is critical to understand the potential of "mobile health" (mHealth) initiatives to impact antenatal and infant health. As part of routine pregnancy surveillance in our trials, pregnant women were interviewed at 1 month postpartum to collect data on complications of labor and delivery. As part of the assessment we analyzed reported use of mobile phones during 611 intrapartum crises occurring between 2007 and 2010. During this time period, reported household ownership of cell phones in our study population increased from 20.4% to 42.5%. This was starkly different by socioeconomic status, where mirrored growth continued with parallel slopes, suggesting a near-constant equity gap during the period of study. During reported obstetric emergencies, 55.2% (n=337) of respondents reported using a mobile phone. More than half (57.0%, n=193) reported using a mobile phone to receive medical advice; 71.7% (n=241) used a mobile phone to call a health care provider, 32.6% (n=110) to arrange for transportation, and 20.9% (n=70) to ask for financial support (categories were not exclusive). In over half of reported intrapartum emergencies, mobile phones were used to request medical care or information or to arrange for medical services. Birth notification efficiency was also studied in families who did and did not own a household mobile phone, in a fixed-line free environment. Interestingly, the rapidity of notification was not impacted by phone ownership. Access to mobile phones for pregnant women and their families, especially during the late and intrapartum period, presents new opportunities to reduce life-threatening maternal complications in poor, rural settings. New windows of opportunity to target interventions to newborns are also created by early labor and birth notification systems made possible with mobile phones.

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INFECTION AMONG DISPLACED POPULATION PORT AU PRINCE: COMPARISON OF EARLY POST QUAKE AND YEAR AFTER EARTHQUAKE PERIODS

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Earthquake in Haiti in January 2010 led to more than 300 000 causalities, mainly due to early trauma and crash syndrome. Only about 30,000 died in the post quake period (10%) due to infectious diseases e.g. infected wounds, pneumonia, and latest, since October 2010 also hypovolemic shock due to cholera. The purpose of this study is to compare spectrum of infectious diseases after quake (January - February 2010) and one year after (2011). Number of patients and spectrum of disease was compared

with univariate analysis with statistical package EPI INFO. First group of 1182 patients from Quisejneia University Hospital has been compared with Field Hospital in February 2011. There was no significant difference in respiratory tract infections and majority of other infectious diseases between the early period and after first year. Only wound infections (33.6% vs. 1.3%; *P*<0.01) and hypertension (15.4% vs. 4.3%; *P*<0.01) has been significantly more frequently observed among patients coming after first month after earthquake (each period) and vice - versa sexually transmitted diseases (14.4% vs. 15%; *P*< 0.01). Wound infections responded with respiratory tract infections majority of infectious diseases (33.6% and 30.2%) followed by hypertension (15.4%) in the early period and respiratory tract infections (37.1%), sexually transmitted diseases (14.9%) and skin soft tissue (13.8%) in the second period, one year after the earthquake.

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GLOBAL ESTIMATES OF SICKLE HEMOGLOBIN IN NEWBORNS

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Reliable estimates of the populations affected by medical conditions are necessary to guide efficient allocation of resources in public health. Despite sickle cell disease being the most common haemoglobinopathy globally, up-to-date estimates of the populations affected are lacking. Moreover, only national estimates of heterozygous (AS) and homozygous (SS) newborns have been published and their precision is not known. Using a georeferenced database of sickle haemoglobin (HbS) surveys, a contemporary evidence-based global map of HbS allele frequency distribution was created within a Bayesian model-based geostatistical framework. This map illustrates strong sub-national spatial heterogeneities and shows high allele frequencies across most of sub-Saharan Africa, the Middle East and India, as well as in areas where the gene spread following human migrations (rather than selection), in Western Europe and along the eastern coast of the Americas. The pairing of predicted HbS allele frequencies with high spatial resolution population counts for 2010 and national crude birth rates enabled calculation of global, regional, national and sub-national estimates of the annual number of AS and SS newborns. The uncertainty in these estimates was calculated using sampling of the allele frequency posterior predictive distributions. In many low- and middle-income countries, the epidemiological transition has greatly reduced infant and child mortality, and improved the survival prospects of HbS patients. In most high-income countries, the need for appropriate diagnoses and genetic counselling to control the number of newborns affected and reduce the risk of complications, as well as the economic burden of treatment and hospitalization, has become more evident. Globally, this situation results in an increasing impact of HbS on public health systems. By taking into account local heterogeneities in HbS allele frequencies and providing uncertainty measures, the maps and estimates presented here provide key spatial intelligence on our current knowledge at various scales and define areas most in need of further research.

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GETTING THE RIGHT DATA IN THE RIGHT PLACE AT THE RIGHT TIME: AUTOMATING THE COLLECTION OF LOGISTICS DATA OF MALARIA PRODUCTS IN ZIMBABWE

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In Zimbabwe, 60% of the population is at risk of malaria. Malaria is the second cause of outpatient consultation, the biggest cause of death of young children, and the cause of almost 20% of reported maternal mortality. The Ministry of Health and Child Welfare (MOHCW) is implementing key interventions in diagnosis, prevention, and treatment of malaria. Central to these interventions is a consistent supply of medicines and rapid diagnostic tests whenever and wherever they are needed. The Zimbabwe Informed Push (ZIP) logistics system manages malaria commodities, along with other medicines and medical supplies. Modeled along the Zimbabwean system which manages family planning and PMTCT commodities, the ZIP system is a delivery truck topping up system, where trucks act as rolling warehouses. Delivery teams are led by District Pharmacy Managers. Every facility in the country receives quarterly deliveries, and are topped up to the maximum stock level, based on their rate of consumption and stock on hand. The implementation of ZIP has resulted in a more than 95% coverage rate, and stockouts of malaria medicines have significantly decreased. Beginning in May 2011, the collection of logistics data is automated. Delivery team leaders carry laptops loaded with a software program - the Automated Delivery/ Receipt Voucher (AutoDRV). After consulting with facility staff, they enter data on stock on hand, and any losses and adjustments that may have occurred. The software calculates the quantity that the facility needs to reach its maximum stock level, and the necessary quantities of products are delivered. Upon completion of a delivery round, Team Leaders return the laptops, and the data they have collected is synched with a central level software tool, Top Up. Aggregated national reports on consumption and stock on hand of key malaria products is available almost immediately after deliveries are completed. Automating data collection has significantly reduced the calculations (and potential errors) that Team Leaders make, and has reduced the amount of time that must be spent at each facility.

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MOLECULAR SCREENING FOR CYTOMEGALOVIRUS (CMV) INFECTION AMONG HIV PATIENTS REGISTERING AT A MAJOR HIV TREATMENT CENTER IN LAGOS, SOUTHWEST NIGERIA

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Cytomegalovirus (CMV) infection can be life threatening for human immunodeficiency virus (HIV) infected patients, organ transplant recipients and neonates. CMV related retinitis is the most common ocular opportunistic infection in these people and often leads to blindness if left untreated, while early diagnosis helps to improve their quality of life. Detection by polymerase chain reaction (PCR) has been very useful in predicting retinitis several months ahead of clinical symptoms. Recently, there have been cases of sudden loss of vision in newly registering HIV patients in the antiretroviral (ARV) clinic of Nigerian Institute of Medical Research (NIMR). Consequently, we utilized PCR to identify CMV positive cases for prompt therapeutic interventions after obtaining ethical approvals from NIMR Institutional Review Board and AIDS Prevention Initiative Nigeria (APIN)/Harvard School of Public Health President's Emergency Plan for AIDS Relief (Harvard PEPFAR) and patients' informed consent. DNA extracted from patient's whole blood was PCR amplified for CMV immediate early (IE) and late (LA) genes. Chi-square and correlation analyses were used to determine relationship between CMV infection, CD4 counts and HIV viral load. Between July and November 2010, 218 HIV patients were screened for CMV infection and 34 (15.6%) were positive; [IE gene = 3 (8.82%); LA gene = 31 (91.18%)]. Statistical analysis showed that 20 (58.82%) of the CMV positive patients had viral load greater than 10,000/ml, showing a positive correlation between CMV and HIV viral load (r = 0.025) but the relationship was not statistically significant ($x^2 = 0.734$; $p>0.05$). Twenty one (61.76%) CMV positive patients also had CD4 counts < 200 showing a positive correlation between CMV prevalence and low CD4 counts (r = 0.613), however the

relationship was not statistically significant ($x^2 = 0.613$; $p>0.05$). All CMV positive cases were referred for further ophthalmologic evaluation and initiation of pre-emptive therapy so as to minimize morbidity and mortality among the patients.

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HOUSEHOLD FOOD SECURITY AND NUTRITION AS A FACTOR FOR ADHERENCE TO ANTIRETROVIRAL THERAPY (ART) AND TREATMENT OUTCOMES AMONG PLWHA IN WOLAITA ZONE, SOUTH ETHIOPIA

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ART is an essential component of care for PLWHA and adherence to ART is strictly required for better outcome. Different factors have been studied as predictors to adherence to ART, but a few studies addressed household food security status as factor for adherence to ART. The objective of this study is to assess the food and nutrition security status of households with PLWHA on ART in Wolaita Zone and to determine its effects on adherence to ART and treatment outcomes. Institution-based cross-sectional study was conducted in Wolaita Zone to assess the household food security and nutritional status of PLHIV on ART in the zone and to determine their effects on adherence and treatment outcomes between December 2009 and February 2010. The study was conducted on 323 PLHIV aged 18 or more selected from two health centers and two hospitals providing ART for more than 100 clients by systematic random sampling. Household food insecurity is found to be a serious problem affecting great majority of PLHIV on ART in Wolaita Zone. Some 93.8% of households with PLHIV on ART were found to have some sort of food insecurity. Proportion of malnutrition is also found to high among study participants affecting some 14.2% of PLHIV after being treated for a median duration of 28 months, suggesting poor response to therapy. 7.7% of the participants admitted missing one or more doses of ART within seven days before interview. Food insecurity was found to have a statistically significant relation with non adherence to ART ($p = 0.025$). Similarly, PLHIV in food insecure households showed little change in BMI as compared to those who came from food secure households ($P<0.001$).The level of food insecurity observed among PLHIV in Wolaita zone is alarmingly high and negatively affecting their health and response to treatment. Integration of nutrition support into routine ART care is widely advocated and efforts are underway to do so in the study locality, but the scale of nutrition support and its equity is far from desired. Organizations providing nutrition care should expand their services and devise strategies to reach out for those hard to reach and most in need.Efforts targeted at enabling PLWHA self-help themselves should also be intensified and strengthened.

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EPIDEMIOLOGICAL, CLINICAL AND HISTOPATHOLOGICAL INVESTIGATIONS ON CUTANEOUS LEISHMANIASIS ASSOCIATED WITH HIV INFECTION IN NORTHERN CAMEROON

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The leishmaniases are a group of vector-borne parasitic diseases caused by protozoa of the genus *Leishmania*. The disease is endemic in the tropics, subtropics and southern Europe. Despite the fact that leishmaniasis is widely reported as an opportunistic infection in HIV-infected individuals, the relationship between the cutaneous form of the disease and HIV infection remains poorly understood. Both HIV infections and cutaneous leishmaniasis (CL) occur in northern Cameroon. However, the association of CL and HIV infection is not documented in the country. Consequently, we conducted epidemiological, clinical and histopathological studies on CL and *Leishmania*/HIV co-infection in northern Cameroon. Of the 32,466 persons were surveyed, 146 (0.5%) were clinically and parasitologically diagnosed with CL lesions and an additional 261 (0.8%) had CL typical scars indicative of past cases. Clinically, the disease ranged from localized to disseminated CL with the number of lesions varying from 1 to 20 per individual. HIV serological testing was carried out on serum samples of all CL active individuals and seven of them (4.8%) were HIV positive. All seven subjects showed antibodies to HIV-1 while two of them were positive for HIV-2. Several parameters such as the number of lesions and lesion sizes were more marked in HIV co-infected individuals as compared to HIV negative controls. In both CL and Leishmania/HIV co-infected subjects, the parasite isolates were identified by DNA sequencing as *L. major*. Immunohistochemical analyses of skin biopsies obtained at different time points showed fewer epidermal Langerhans cells, CD1a+ dermal dendritic cells, CD68+ macrophages, as well as fewer CD4+ T cells and CD20+ B cells in HIV co-infected individuals. HIV co-infected patients also showed reduced degranulation of skin mast cells in CL lesions. Analysis of the cytokine profile is underway and will provide a better picture of CL and HIV co-infections in humans. This is the first report of *L. major*/HIV co-infection in Cameroon and Central Africa. A detailed understanding of the immunological responses in *Leishmania*/HIV co-infected individuals is important for the development of optimized therapeutic regimens for this severely affected group. Our findings provide important data for the development and implementation of successful control programs against CL and HIV co-infection in this geographical area.

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EVIDENCE-BASED UPDATE ON THE OPTIMAL TIME FOR INITIATION OF ANTIRETROVIRAL THERAPY (ART) IN PATIENTS WITH HIV INFECTION AND CONCURRENT PULMONARY TUBERCULOSIS (TB): A SYSTEMATIC REVIEW OF RANDOMIZED CONTROLLED TRIALS

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Currently, initiation of HIV therapy is based on CD4 cell count. However, the point during the course of HIV infection at which ART is best initiated in patients with concomitant pulmonary TB remains unclear. Guidelines issued by various agencies provide different initiation recommendations according to resource availability. The aim of this systematic review was to provide an evidence base from which health care providers can make effective decisions in treating patients. We searched the following databases from January 1980 to February 2011: PUBMED, EMBASE, and WHO International Clinical Trials Registry Platform, AEGIS database for conference abstracts, the Cochrane Central Register of Controlled Trials, and the Cochrane Database of Systematic Reviews. A total of 63 full text articles were identified and supplemented by a bibliographic search. Two review authors independently assessed study eligibility, extracted data, and graded methodological quality and bias. Three eligible randomized controlled trials were included (N = 1393). In our pooled analysis, we combined the clinical data for both trials comparing early initiation ART (less than four weeks after starting anti-TB treatment) versus delayed initiation of ART (four weeks or more after starting anti-TB treatment). There was a 52% decrease in AIDS progression/Death (RR=0.48, 95% CI [0.28, 0.84], p= 0.01) in the group with early initiation of ART (n/N =104/823) compared to the group with delayed initiation of ART (n/N=148/570). There was no evidence of heterogeneity or publication bias. This systematic review shows that there is sufficient evidence in support of early initiation of ART in HIV infected patients with concurrent pulmonary TB. We therefore recommend initiation of ART within 4 weeks of pulmonary TB diagnosis and treatment in HIV patients.

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EXAMINING THE EFFECT OF SEVERE BACTERIAL INFECTIONS ON SURVIVAL IN A COHORT OF HIV-INFECTED CHILDREN AT A PEDIATRIC HIV CARE FACILITY IN LILONGWE, MALAWI

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HIV infections still pose a serious problem in the developing world with countries in sub-Saharan Africa contributing 67% of the 33 million infections worldwide. In Malawi, a million of the total population (14 million) are living with the AIDS causing virus. So far the provision of antiretroviral therapy (ART) has focused on adults, with children making up less than 10% of those on treatment in September 2009. Studies on HIV/AIDS have concentrated on the older population with little researched on the survival of children on ART. Children are known to be typically prone to severe bacterial infections (SBIs) such as pneumonia, sepsis, meningitis, and tuberculosis (TB) among others, even in the absence of HIV infection in the early stages of life. Longitudinal data for a cohort of children started on ART provides an opportunity to explore how the survival in children with a history of SBIs compares to that of children who have never had these infections. Survival analysis methods were applied to data from a cohort of HIV infected children aged 15 years or less receiving care at Baylor Children's Foundation pediatric HIV care clinic in Lilongwe, Malawi registered between October 2004 and July 2010. Factors that are potentially predictive of outcome will be explored and reported. Methods for handling missing data for prognostic factors will also be explored.

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REMOVAL OF ONE OR MORE HIV/AIDS RELATED INFECTIONS BY USING PLANTS: FINDINGS FROM A RAPID ASSESSMENT STUDY IN SATKHIRA DISTRICT OF BANGLADESH

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Bangladesh is at risk of an HIV/AIDS epidemic. This is due to the high prevalence of the disease in neighbouring countries and the limited access to counselling and testing services on account of social stigma. Traditional health practitioners (THPs) of Bangladesh primarily use plants for treatment of various ailments. The selection of plant is a closely guarded secret and is usually kept within the family. As a result, the use of plants varies widely between THPs of different areas within the country, and is based on both plant availability and the THPs' unique knowledge derived from practice. The present study was to conduct a survey amongst the THPs to learn more about the plants used to treat one or more HIV/AIDS related infections like tuberculosis, diarrhoea, vomiting, tumours, sexually transmitted diseases, and fevers in the Satkhira district of Bangladesh. This area is unique in its proximity to the Sunderbans forest region and contains quite different plants from other parts of the country because of high salinity in the soil and water. Semi-structured questionnaires were administered to twenty-four THPs to evaluate the THPs' perceptions and practice relating to causation and treatment of one or more HIV/AIDS related infections. The THPs described the signs, symptoms, and cause of one or more HIV/AIDS related infections. Details of the preparation and use of plants for management of one or more HIV/AIDS related infections were recorded. Plant specimens were collected and identified at the Bangladesh National Herbarium. In the present study, forty-one plants belonging to thirty-nine genera and twenty-eight botanical families were found to be used to treat one or more HIV/AIDS related infections in Satkhira district of Bangladesh. It was further noted that most THPs use a single plant or plant part to treat a single ailment. Information on traditional medicinal uses of mangrove plants is scant in the scientific literature. From that view point, scientific studies conducted on the plants may lead to discovery of more effective drugs than in use at present.

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IMPACT OF HIV-1 INFECTION ON PERFORMANCE OF TWO MALARIA RAPID DIAGNOSTIC TESTS (MRDTS)

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The performance of two histidine-rich-protein-type-2-based (HRP-2) malaria rapid diagnostic tests (mRDTs) was examined in a rural area with high prevalence of malaria and HIV-1 infection in 113 and 435 febrile patients aged ≥15 years with and without HIV-1 infection, respectively. Febrile patients presenting to Lirangwe Health Center were tested for HIV-1 infection (Unigold test) and for *Plasmodium falciparum* parasitemia with both mRDTs (Bioline SD RDT and ICT Diagnostics RDT) and microscopy. The sensitivity and specificity of mRDTs in HIV-positive and HIV-negative patients was assessed using microscopy as the gold standard. Bioline SD had a sensitivity of 94% versus 97% (p=0.4) and specificity of 51% versus 47%, (p=0.6) for any parasitemia while ICT diagnostics had sensitivity of

94% versus 97%, (p=0.4) and specificity of 51% versus 50%, (p=0.9) in patients with and without HIV-1 infection respectively. HIV-1 infection does not appear to affect the performance of these HRP-2-based mRDTs.

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HIV CO-INFECTION IN MALAWIAN CHILDREN WITH CEREBRAL MALARIA

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Malaria and HIV co-exist in many malaria-endemic areas, but there have been very few studies of their interaction in children with severe and complicated malaria. Among patients who meet the standard clinical case definition of cerebral malaria (CM), those with "true" cerebral malaria can be identified more accurately when one or more features of malaria retinopathy are observed in the course of an ocular funduscopic examination. We studied the impact of HIV co-infection in children with retinopathy -positive cerebral malaria in Blantyre, Malawi. A retrospective analysis of 1,152 children, admitted to an ongoing research study between 1996-2010, with known HIV status, and who had undergone ophthalmoscopic examination within six hours of admission was carried out. Seven hundred fifty were retinopathy-positive, and of these, 111 (14.8%) were also HIV-positive. The median age (50 months) of HIV-positive patients with retinopathy-confirmed CM was higher than that of HIV-uninfected patients (33 months, p <0.01). Survival rates did not differ between the two groups, but median coma duration times were longer in the HIV-positive CM patients (42 hours versus 34 hours, p=0.02). There were no differences in anthropometric measurements, parasitemia, hematocrit, hematologic findings including platelet count and white cell count, blood glucose and lactate concentrations on admission, parasite clearance times, specific features of malaria retinopathy, rates of blood culture positivity or rates of neurological sequelae in survivors one month after admission. We conclude that, among children with retinopathy positive cerebral malaria, HIV has a slight but discernible impact on presentation and progression of disease and recovery, but does not have an impact on outcome.

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PREVENTION OF MOTHER TO CHILD TRANSMISSION SERVICES IN PRIVATE HEALTH FACILITIES IN WAKISO DISTRICT, UGANDA

Maternal to child transmission (PMTCT) is the second commonest mode of HIV transmission, after sexual transmission. Uganda has been implementing PMTCT for 10 years. However population coverage for services has remained low. The study aimed at assessing the contribution of private facilities to PMTCT service delivery. This study was undertaken t assess the level of PMTCT service delivery in private health facilities in Wakiso district. This was a cross-sectional study. A check list was used to collect both qualitative and quantitative data. An interview was held with each in charge of the health facility followed by physical inspection of the PMTCT infrastructure within the facility. In the 10 health facilities assessed, on average in a month 50 HIV tests are done, 100 women attend ANC and 120 mothers deliver form these facilities. Eight out of ten facilities offered HIV counseling and testing. Only three facilities offered PMTC services and the other seven, referred mothers to other units for PMTCT services. Facilities that offered PMTCT services reported frequent stock outs and were not sure of sustainability plans. Family planning and STDs services were present in 80% of the units. Very few providers (20%) had ever received training in PMTCT, Early Infant Diagnosis (EID) or HCT. None of the health units had PMTCT policy and guidelines in place. In conclusion, PMTCT service coverage in private facilities is still very low yet a good number of women (120) deliver in these facilities. Support in terms of test kits, PMTCT drugs, trainings and supervision is highly recommended.

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RELATIONSHIP BETWEEN ASEXUAL MALARIA PARASITE DENSITY AND AGE, PCV, WBC AND ESR IN HIV-INFECTED PATIENTS IN NORTH CENTRAL NIGERIA

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Infections by malaria parasite and human immunodeficiency virus (HIV) represent major public health problems in many parts of the world. Both infections kill millions of people each year and both infections are scourges of developing nations in Africa, India, southeast Asia and South America The relationship between asexual malaria parasite density and age, PCV and ESR in HIV-infected individuals was examined. Routine investigations included Random blood sugar (RBS) and serum electrolytes. Haematological profile included Packed Cell Volume (PCV), total white blood cell count (WBC), and erythrocyte sedimentation rate (ESR). Parasite density was determined by microscopy. Fifty-nine out of 78 (75.6%) HIV-infected patients had levels of asexual parasite density (ap/l) < 500 ap/l of whole blood. Eleven (14.1%) had levels of parasitaemia 500-1000 ap/l of whole blood while 7(8.97%) had 1001-5000 ap/l of whole blood. Two patients (2.56%) had parasite density of 5000 ap/l and above. Parasitaemia was more elevated in the 26-35 year olds (48.7%) followed by those aged between 36-45 years (19.2%). There was no significant difference (P>0.05) in asexual parasite density between the age groups ($Cal^2 = 6.87 < Tab^2_{0.05}$ $df_{15} = 25.00$). It was observed that 27.9 % (17/61) of the patients had leucopaenia (WBC count $\leq 4.0 \times 10^9$/l). All patients with leucopaenia had malaria parasitaemia. In relation to asexual parasite density, slightly low PCV levels of 15-30% was observed in 23.8% (15/63) of the patients although no significant difference (P>0.05) was observed between this level and higher PCV levels ($Cal^2 = 0.9729 < Tab^2_{0.05}$ $df_6 = 12.59$). Elevated mESR (>20 mm/hour) was recorded in 17.8% (20-40mm/hour) and 51.1% (>40mm/hour) of the patients with asexual parasite density. The presence of HIV could have contributed considerably to the elevated ESR observed among the patients, since many of the HIVSP patients with raised ESR had low parasite density and normal PCV levels.

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IN VITRO SENSITIVITY OF *PLASMODIUM FALCIPARUM* FIELD ISOLATES TO EXTRACT FROM CAMEROONAIN ANNONACEAE PLANTS

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In a search for new plant-derived antimalarial extracts, 19 fractions were obtained from three Annonaceae species, *Uvariopsis congolana*, *Polyalthia oliveri*, and *Enantia chlorantha* with yields ranging from 0.33% to 4.60%. The extracts were prepared from 500g of each plant part, using organic solvents to afford 5 methanolic fractions (acetogenin-rich), 5 water fractions, 5 hexane fractions, and 4 interface precipitates. Evaluation of the activity of fractions *in vitro* against field isolates of the malaria parasite *Plasmodium falciparum* showed that acetogenin-rich fractions and interface precipitates were the most potent, with IC_{50} values ranging from 0.05 µg/ml to 8.09 µg/ml. Sensitivity of parasite isolates to plant extracts varied greatly, with over 100 fold difference from isolate to

isolate in some cases. The active acetogenin-rich fractions and interface precipitates were assessed in combination with chloroquine in the same conditions, and showed additive interaction in the huge majority of cases. Synergistic interactions were found in some cases with acetogenin-rich fractions. Acute toxicity of promising fractions was evaluated through oral administration in Swiss albino mice. Tested fractions appeared to be safe, with LD_{50} values higher than 2g/kg. In summary, acetogenin-rich fractions from Annonaceae species showed high potency against *P. falciparum* field isolates and safety by oral administration in mice, supporting their detailed investigation for antimalarial drug discovery.

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PHARMACOKINETIC EVALUATION IN A RANDOMIZED CONTROLLED TRIAL OF INTRAVENOUS ARTESUNATE IN ADULTS WITH UNCOMPLICATED MALARIA IN KENYA

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The pharmacokinetics (PK) of cGMP intravenous artesunate (AS) were evaluated after a single dose of 2.4 mg/kg infused over two minutes in 28 adults with uncomplicated *Plasmodium falciparum* malaria. Plasma concentrations of AS and dihydroartemisinin (DHA), an active metabolite of AS, were measured using a validated LC-MS methodology. Pharmacokinetic data were analyzed with a model-dependent finding for AS and a model-independent finding for DHA. After intravenous infusion, the concentration of the parent drug rapidly declined, and the AS was rapidly converted to DHA. AS and DHA showed a mean elimination half-lives of 0.17 h and 1.25 h, respectively. The high mean peak concentration (C_{max}) of AS was 28,962 ng/ml while the C_{max} of DHA was 2,853 ng/ml. A significant variability was noted in the PK profiles of the 28 patients tested. For example, C_{max} values of AS calculated ranged from 3,362 to 159,822 ng/ml and 477 to 6,434 ng/ml for DHA. The mean AUC of AS was approximately half that of DHA (1,878 ng·h/ml vs, 3,587 ng·h/ml). The rapid conversion of AS into DHA suggests that former is a prodrug, but in the present trial the DHA/AS ratio observed were 2.5 during the one day single treatment. The AUC and half- lives of DHA measured were significantly larger and longer than for AS. However, intravenous AS can provide 10-fold higher peak concentrations of AS than DHA, supporting the belief that AS has intrinsic antimalarial properties. This may be crucial for the rapid elimination of parasites in patients with severe malaria.

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NANOEMULSION COMPARED TO SESAME OIL AS VEHICLE FOR SUBCUTANEOUS DELIVERY OF ARTEETHER IN TREATMENTS OF MICE INFECTED WITH *PLASMODIUM BERGHEI* MALARIA

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Previous pharmacokinetic studies showed arteether (AE), an oil-soluble artemisinin, induced slower killing of malaria parasites due to the formation of a drug depot at the intramuscular injection sites, which is associated with fascia inflammation in muscles. In order to increase the absorption rate and efficacy of AE, a new nanoemulsion formulation of AE was made using an ultra-sonicator and the pharmacokinetics of emulsion and sesame oil formulations of AE were compared. The mean particle size of the emulsion formulation was measured by using a LA-950 laser particle size analyzer, showing a particle size range from 0.274 to 0.351 μm. Following infection of ICR mice with 5×10^6 *Plasmodium berghei*-infected erythrocytes intraperitoneally on day 0, the animals were treated with the nanoemulsion and sesame oil formulations of AE at 25 mg/kg daily dosing on days 3 to 5 post-infection. The experimental endpoint of efficacy was flow cytometric determination of malaria blood parasitemia in the infected mice over a 30 day period. The parasitemia curve in the initial period after dosing showed a significant difference in the duration of the lag phase from 5.87 hr in the animals treated with the AE nanoemulsion to 8.22 hr in the mice treated with the AE sesame oil formulation. Similarly, rapid parasite clearance was noted with a mean PC_{50} time of 13.24 hr in mice after dosing with the AE nanoemulsion, which was significantly shorter than to the parasite clearance observed in animals treated with AE sesame oil (PC_{50} = 18.67 hr). Subcutaneous dosing of AE dissolved in sesame oil was neither able to exterminate parasites rapidly, nor was this formulation capable of reducing parasitemia as well as the AE nanoemulsion. In summary, we have shown that the AE nanoemulsion was found to be the more effective formulation in terms of particle size reduction, pharmacokinetics, and parasite killing.

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PROPHYLACTIC ACTIVITY OF ORAL TAFENOQUINE IN LIVER STAGE OF A RODENT MALARIA MODEL BY USING A REAL-TIME *IN VIVO* IMAGING SYSTEM

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Malaria remains an important cause of global morbidity and mortality. As antimalarial drug resistance escalates, new safe and effective medications are necessary to prevent and treat malaria infections. WRAIR is developing tafenoquine (TQ), an analogue of primaquine, which is expected to be effective in both preventing and treating malaria in deployed military personnel. TQ has a long half-life of 14 days and is generally safe and well tolerated, although it cannot be used in pregnant women and individuals who are deficient in the enzyme glucose-6-phosphate dehydrogenase. A transgenic *Plasmodium berghei* parasite expressing the bioluminescent reporter protein luciferase was utilized to visualize and quantify parasite development using a real-time *in vivo* imaging system (IVIS) in live C57BL/6 Albino mice. Luciferase-expressing sporozoites isolated from the same batch of mosquitoes were inoculated into mice on the same day to control for biological variability in sporozoite preparations. Each mouse was inoculated intravenously in the tail vein with approximately 50,000 sporozoites suspended in 0.1 ml volume on day 0. Daily dosing of TQ was conducted for 3-consecutive-days (-1, 0, and 1 day after inoculation) in the assessment. Parasitemia in the blood stage was also monitored by a flow cytometry method. Compared to vehicle control animals, mice treated with ≥ 10 mg/kg TQ demonstrated no bioluminescence signal, and the TQ-treated mice also showed 100% inhibition of luciferase-expressing sporozoites in the location of the liver. At the 5 mg/kg TQ dose level, 87% suppression of signal was shown at the 24 hour measurement and 100% suppression of signal was observed thereafter in all mice. Only partial cures were observed in the animals treated with a TQ dose of 2.5 mg/kg In summary, we have shown that TQ is highly effective for causal prophylaxis in *P. berghei* infected mice with protective efficacy at a minimal curative dose of 5 mg/kg daily for 3 days, which is a dose 3-4 times lower than a similar dose of primaquine in this IVIS model.

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STAGE-SPECIFIC SENSITIVITY OF *PLASMODIUM BERGHEI* TO ARTESUNATE AND OTHER ANTIMALARIAL DRUGS IN C57BL/6 MICE

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There is an urgent need for basic studies focusing on the development of new drugs and vaccines against malaria, which remains a disease of significant morbidity and mortality around the world. The effects of drugs and vaccines on parasite growth are often investigated *in vitro*

or *in vivo* by focusing on how well these products work in different stages of the malaria life cycles. Recently, we established a new method for determining the malaria parasite life-cycle by flow cytometry (FCM) in our laboratory. This method is capable of quantitating merozoites, ringforms, early trophozoites, later trophozoites, immature schizonts, and mature schizonts. FCM demonstrated a clear separation in fluorescent distribution between each stage and this method compared well with traditional microscopy. The inhibitory effects of artesunate, mefloquine and pyrroloquinazolinediamine against parasite growth in different life stages were examined in *Plasmodium berghei* infected mice with this new FCM method following single intragastric dose of each drug were administrated to each mouse on day 8 post-infection. The stage-specific results shown by the FCM method demonstrated that mefloquine has a mode of action on mature parasite forms, while artesunate and pyrroloquinazolinediamine were rapidly effective against immature (ring and trophozoite) and also mature parasite forms (schizont and merozoite) in mice. The potency of each antimalarial drug differs, and the FCM method showed pyrroloquinazolinediamine to be more potent than artesunate which in turn was more potent than mefloquine. This study demonstrates a sensitive method to conduct malaria life cycle stage-specific drug sensitivity studies *in vivo*, which provides researchers a new tool to examine and compare prospective antimalarials. Further improvements and assay qualification studies are planned for this *in vivo* life-cycle stage assay in animals to demonstrate the utility of this method for use in malaria drug and vaccine research.

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NANOPARTICLE PREPARATION OF VARIOUS POORLY SOLUBLE NOVEL ANTIMALARIAL COMPOUNDS WITH A HIGH PRESSURE HOMOGENIZER

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The formulation of poorly water- or lipid-soluble compounds has always been a challenging problem faced by research scientists. At WRAIR, more than 50% of new compounds synthesized as potential antiparasitic drugs are poorly soluble. Nanoparticle formulation is a simpler formulation strategy to improve solubility when compared to other techniques. Our nanoparticle formulation approach based on high pressure homogenization performed in water or solvent medium. We have been able to generate nanoparticle crystal drug with greater hardness at a size ranging from 0.25-0.40 µm under production conditions of 1500-3000 bars in 5-10 homogenization cycles. Crystal drug with less hardness can be generated into particles of smaller size ranging from 0.70-1.20 µm. An antiparasitic drug called decoquinate, is an amorphous compound, was dispersed in a crystalline compound first and then mixed with a stabilizing agent to perform the homogenization step at a pressure between 500 and 750 bars for 70-100 homogenization cycles. The final particle size of this nanoparticle decoquinate was in the range of 0.45-0.95 µm. For a harder compound such as WR299666, a glass grinder method was used to manually decrease particle size to a diameter of 42.2 mm. This compound was treated with an ultra-sonicator methodology employing evenly distributed pulses of sound to break apart the ground particle to further decrease particle size of 1.5 mm. Further treatment of WR299666 with a high pressure homogenizer at 2500 bar for 6 homogenization cycles reduced the particle of this drug to an average size of 0.48 mm producing a drug nanosuspension. Through these two examples we show the parameters determining the final nanoparticle size achieved include the power density (homogenization pressure), number of homogenization cycles and the innate hardness or softness of the drugs. These techniques can be used, to reduce particle size of novel chemical entities to significantly decrease particle size and improve oral bioavailability.

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NOVEL AMINOINDOLE INHIBITORS OF *PLASMODIUM FALCIPARUM*: A CANDIDATE IN PRECLINICAL DEVELOPMENT

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The aminoindole Genz-668764 is an analog of Genz-644442 which was originally identified as a hit in a high throughput live-dead screen of the Broad small molecule library against *Plasmodium falciparum*. Genz-668764 is a single enantiomer with IC_{50} values of 65 and 28 nM against *P. falciparum* strains Dd2 and 3D7 respectively. Against clinical field isolates from Senegal, the IC_{50}'s ranged from 20 to 55 nM; data is also presented for additional laboratory strains. Studies reported previously, showed that Genz-668764 cured animals infected with *P. berghei* N-clone, with an ED_{50} of 32 mg/kg/day when dosed 4 days b.i.d.; 2/5 animals infected with *P. berghei* ANKA strain were cured dosing at 100 mg/kg/day; ED_{50} against the ANKA strain was 26 mg/kg/day. In preliminary rat 7-day safety studies, the only significant clinical finding was a reduction in the rate of weight gain in animals receiving the highest dose (300 mg/kg/day). Based upon the safety and efficacy data, Genz-668764 has been moved into preclinical development and is currently being studied in a 14-day rat study at 30, 100 and 300 mg/kg/day. After cessation of dosing, a subset of animals were allowed to recover for 2 additional weeks. Body weight, clinical pathology, a functional observational battery assessment and toxicokinetics were assayed. Data for these is presented. In addition, we have determined the parasite reduction ratio (*in vitro*) which shows that Genz-668764 kills rapidly, similar to chloroquine. Taken together, Genz-668764 appears to be a promising candidate for further development.

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GENZ-669178: A NOVEL INHIBITOR OF *PLASMODIUM FALCIPARUM* DIHYDROOROTATE DEHYDROGENASE IS A CANDIDATE FOR PRE-CLINICAL DEVELOPMENT AS AN ANTI-MALARIAL AGENT

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Plasmodium falciparum is unable to salvage pyrimidines and must rely on *de novo* biosynthesis of these crucial biomolecules for survival. Dihydroorotate dehydrogenase (DHODH) represents a viable target for chemotherapeutic development, catalyzing the rate-limiting step in the *de novo* pyrimidine biosynthetic pathway in which dihydroorotate is formed through a coupled redox reaction utilizing a mitochondrial respiratory chain ubiquinone. A series of 5-benzimidazolyl-N-alkylthiophene-2-

carboxamides has previously been described as exhibiting potent and selective inhibition of plasmodial DHODH that was well correlated with *in vitro* potency against the *P. falciparum* 3D7 and Dd2 parasites. Genz-669178 has emerged as the lead compound in this series, demonstrating low nanomolar IC_{50} values against the enzyme and the parasite, activity against exoerythrocytic stages, and ED_{50} values in several murine models of 8-21 mg/kg/day with oral b.i.d. dosing. The compound possesses a favorable metabolite profile, excellent chemical stability, and a parasite reduction ratio that is intermediate in comparison to a panel of known anti-malarials. Preliminary *in vivo* rat toxicology studies have established the no adverse effect limit (NOAEL) at >600 mg/kg/d, with a predicted human therapeutic margin of >5.5. Genz-669178 is currently being proposed as a candidate for pre-clinical development and progression to first-in-human trials, either alone or in combination with other agents.

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RELATIVE BIOAVAILABILITY OF A FIXED COMBINATION TABLET FORMULATION OF AZITHROMYCIN AND CHLOROQUINE (AZCQ) IN HEALTHY ADULT SUBJECTS

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The combination of CQ and azithromycin (AZ) has demonstrated enhanced efficacy even in resistant strains of *Plasmodium falciparum in vitro* and *in vivo*. This was an open-label, randomized, single-dose, parallel-group study to estimate relative bioavailability of two AZCQ fixed dose combination tablets each containing 250 mg AZ base/155 mg CQ base (Test Treatment), compared with co-administration of individual tablet of 500 mg AZ base and 300 mg CQ base (Reference Treatment) in forty healthy male or female subjects (18-55 years old; body weight > 50 kg). Fasting subjects were randomized (1:1) to receive either Test or Reference Treatment. Subjects were confined to the Clinical Research Unit for two days following drug administration with additional clinic visits on Days 3-5. Blood samples for determination of serum AZ and plasma CQ concentrations were collected at specified time points post dose for noncompartmental pharmacokinetic (PK) analyses. PK exposure parameters of AUClast (area under concentration-time curve from time 0 to time of the last quantifiable concentration) and Cmax (maximum concentration) were calculated. Relative bioavailabilities of log-transformed AUClast and Cmax were analyzed using a one-way ANOVA to estimate the ratio of adjusted geometric means (Test/Reference). Safety evaluations included monitoring of adverse events, clinical laboratory tests and vital signs. All subjects completed the study. Cmax values for the two AZCQ tablets were approximately 13% higher for AZ and 11% lower for CQ compared with Reference Treatment. AUClast of AZ and CQ for the two AZCQ tablets was comparable to the Reference Treatment. The relative bioavailabilities (90% CI) for the two AZCQ tablets were 101% (85.40%-119.11%) for AZ and 99% (83.96%-117.08%) for CQ compared with the Reference Treatment. Both treatments were well tolerated. The AZCQ formulation is being currently evaluated in Phase 3 intermittent preventive treatment for malaria in pregnancy (IPTp) clinical trials.

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PLASMODIUM FALCIPARUM CLPQ PROTEASE, A NOVEL DRUG TARGET FOR MALARIA: A HIGH-THROUGHPUT SCREEN TO IDENTIFY POTENTIAL ANTI-MALARIAL LEADS

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In view of wide spread and evolving resistance in the malaria parasite to current therapeutics, there is a continuing need to identify new drug targets and develop new anti-malarial drugs. The ClpQY protease complex is identified as a novel drug target which is functionally important for blood stage parasite survival, with highly conserved homologs across multiple species of *Plasmodia*. ClpQY (HslVU) is a prokaryotic proteolytic complex which is not present in the human host, consisting of the ClpQ threonine protease and ClpY ATPase. A homology model of *Pf*ClpQ has been constructed and the substrate binding cavity has been elucidated. A small peptide corresponding to the C-terminus of ClpY can disrupt the ClpQ-ClpY interaction; treatment of *in vitro* parasite cultures with this peptide caused significant growth inhibition and resulted in developmental arrest of blood stage parasites. We describe in detail an *in vitro* HTS for inhibitors of ClpQ designed to monitor the release of 7-amido-4-methylcoumarin from the peptide substrate, AMC-LLVY. A standard control inhibitor, MG132 (IC_{50} = 92 μM) was used to monitor assay performance. A small library of 7,000 compounds compiled from the Genzyme and Broad Institute collections was screened, and 91 compounds were identified as hits (50-99% inhibition at 10 μM; 1.4% hit rate). Several novel chemical scaffolds have been identified as potential leads for a medicinal chemistry campaign to design new anti-malarials.

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IN VITRO CYTOTOXICITY OF CURRENT AND EXPERIMENTAL ANTIMALARIAL DRUGS

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An *in vitro* cytotoxicity assay has been developed and used to screen 20 current and experimental antimalarial drugs representing three major classes; sesquiterpene lactones, hemozoin inhibitors and antifolates as well as synthetic and natural compounds. Three mammalian cell lines were used; human liver HepG2, human kidney HEK293 and rodent kidney BHK. In the assay, cells were plated in 96 well microplates at low cell density for 24 h prior to 72 h of exposure to 12 different drug concentrations. Growth inhibition due to cytotoxicity was determined using the alamarBlue cell growth assay. The assay uses resazurin (7-hydroxy-3 H-phenoxazin-3-one 10-oxide) which is a REDOX indicator that is reduced by mitochondrial enzymes to a fluorescent product that is proportional to the number of metabolically active cells. In the present study, the sesquiterpene lactones and hemozoin inhibitors produced data of a sigmoidal growth inhibition curve on a semi-log plot from which IC_{50} values were derived. This was in contrast to antifols where growth inhibition curves showed a more linear response, presumably due to their different modes of action. The cytotoxicity values of different chemical classes of antimalarial drugs were similar for the two human cell lines tested than the rodent cell line. Overall, prodrug cytotoxicity, (eg. proguanil and artesunate) gave consistently higher IC_{50} values than the active metabolite (eg. cycloguanil and dihydroartemisinin). The results can also be used in combination with *in vitro* parasite drug assays to calculate a ratio of IC_{50} mammalian cell culture / IC_{50} parasite culture, also known as a Selectivity Index (SI). A high SI suggests the drug may be well tolerated and efficacious *in vivo*, and vice versa. Therefore the SI can provide useful information prior to preclinical investigations in animal models. Complete results and growth inhibition curves will be presented.

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NEW ANTI-MALARIA SUBSTANCES FOR INHIBITING MEFLOQUINE-RESISTANT *FALCIPARUM* MALARIA

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Malaria caused by *Plasmodium falciparum* is a major cause of global morbidity and, in rare cases, mortality. Now it becomes resistant to various antimalaria especially mefloquine. GAPDH is an essential plasmodial protein and as such is a candidate as an antimalaria drug target. In this study, determining four antimalaria inhibitors directed against this target was studied. The glyceraldehydes-3-phosphate dehydrogenase (GAPDH) recombinant protein from six mefloquine resistant and four mefloquine sensitive *P. falciparum* were produced by ligating in pGEM-T® easy and pET 15b vectors;then, they were transformed in NEB 5-alpha and JM 109 *E. coli* competent cell respectively. They have been solved and compared to the equivalent enzyme from human GAPDH. Subsequently, they were tested against four inhibitors consisting Dyhydroxyacetone (DHA), putative AEBSF molecule ferriprotoporphyrin IX. And Methylglyoxal respectively. This study showed that the inhibition was not different between mefloquine resistant and four mefloquine sensitive parasites. By studying kinetic assay to determine the Steady-State Kinetic Data; *Km*, *Ki* and *Kcat* of Dihydroxyacetone, putative AEBSF molecule, ferriprotoporphyrin IX and Methylglyoxal were 70.02 ± 0.19 %, 39.92 ± 0.16 %, 30.12 ± 0.39 % and 17.68 ± 20.12 % respectively. The first and third ones were not inhibit human GAPDH and these inhibitors bind preferentially to malaria enzyme over human forms. This study showed that it may be possible to develop inhibitors that are reactive against *falciparum* malaria.

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A NOVEL ANTIMALARIAL COMPOUND, JPC2583 WITH POTENT ANTIMALARIAL ACTIVITY *IN VITRO* AND *IN VIVO* AND DOES NOT INDUCE DORMANCY IN *PLASMODIUM FALCIPARUM* PARASITES

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Plasmodium falciparum parasites treated *in vitro* with artemisinin compounds undergo a growth arrest or dormancy, a recently described phenomenon, thought to be responsible for parasite recrudescences and treatment failures in malaria patients following monotherapy with artemisinin derivatives. Furthermore, it has been suggested that dormancy, as a parasite survival mechanism, may also contribute to the development of artemisinin resistance in the field. Therefore, it is of utmost importance to appropriately select the partner drugs for use in combination with artemisinins, so that treatments with these drugs would not result in dormancy and ideally prevent the parasites' recovery from artemisinin-induced dormancy. In this study, a novel antimalarial compound, JPC2583 (patent application in progress), developed by Jacobus Pharmaceutical Co., Inc., that shows potent antimalarial activity *in vitro* and *in vivo* in the rodent-*P. berghei* model, has been tested for its potential to produce dormant parasites, as well as to prevent recovery from dihydroartemisinin (DHA)-induced dormancy. JPC2583 and DHA treatments appear to have different effects on parasites *in vitro*. As shown previously, DHA treatment of *P. falciparum* ring stage parasites at 200 ng/mL (~ 100-fold IC_{90}) for 6 h rapidly halts parasite development, resulting in death for majority of parasites, with a small proportion becoming dormant. These dormant parasites usually resume their growth after 3-5 days. By contrast, ring stage parasites treated with 50- or 100-fold IC_{90s} of JPC2583 were halted in their development at later ring early trophozoite stages and appeared to be morphologically different to those treated with DHA. There were no dormant rings observed upon treatment with JPC2583. Furthermore, JPC2583 treated parasites did not resume their growth up to 4 weeks after starting treatment. Studies of the effects of JPC2583 on the recovery of parasites from DHA induced dormancy are underway and results will be presented.

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PROLONGED SELECTION OF *PFMDR1* POLYMORPHISMS AFTER TREATMENT OF *FALCIPARUM* MALARIA WITH ARTEMETHER-LUMEFANTRINE IN UGANDA

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Artemisinin-based combination therapies (ACTs) are recommended for treating uncomplicated *falciparum* malaria. Partner drugs eradicate persisting parasites, but may select for resistance after the short-acting artemisinins have been cleared. Systematic studies on the duration of selection by ACTs are lacking. Sensitivity to multiple drugs is impacted upon by polymorphisms in the *Plasmodium falciparum* multi-drug resistance (*pfmdr1*) *gene*. To determine the duration of selection by artemether-lumefantrine (AL), we compared the prevalence of key *pfmdr1* alleles between pretreatment isolates and those that emerged after treatment with combination antimalarial regimens in a cohort of children (aged 1-10 y at enrollment) in Kampala, Uganda, followed between 2004 and 2008. Infections that emerged soon after treatment with AL, but not artesunate-amodiaquine (AS+AQ) or amodiaquine-sulfadoxine-pyrimethamine (AQ+SP), were more likely to contain the *pfmdr1* 86N, 184F, and 1246D alleles. Remarkably, despite the short half lives of artemether (~1 h) and lumefantrine (3-4 d), the selective pressure of AL persisted for an extended period. Notably, the prevalence of the 86N, 184F, and 1246D alleles increased from 8%, 9% and 15% before treatment to 77%, 30% and 79%, respectively, in parasites that emerged within 30 days after treatment, and significant selection persisted for 6-8 weeks. Additionally, within 120 days after treatment with AL, the prevalence of the 86N/184F/1246D haplotype, which has been associated with decreased *in vitro* drug sensitivity and with recrudescence after AL treatment, was significantly higher (17%) than the pretreatment prevalence (8%) or the prevalence in the AS+AQ (3%) and AQ+SP (5%) treatment arms. Thus, treatment with AL selected strongly for polymorphisms associated with decreased sensitivity to both components of the drug, and this selective pressure persisted far beyond the half lives of both components, suggesting that, as AL is increasingly used to treat malaria, parasites with diminished sensitivity to the drug will commonly be selected.

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STUDY OF STAGE-SPECIFIC IC_{50} OF QUININE IN FOUR *PLASMODIUM FALCIPARUM* STRAINS USING FLOW CYTOMETRY

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Quinine has long been used as the primary therapy for severe malaria. In recent years, the artemisin derivatives have now become the drug of choice for severe malaria based on several trials which showed superiority

to quinine. However, due to its availability, IV quinine is still used by many developing countries to treat severe malaria. The mechanism of action of quinine remains a mystery to the scientific community. It is thought to act at the level of heme polymerization similar to chloroquine. However, resistance to chloroquine developed at a much faster rate than resistance to quinine, indicating that the drugs may act in dissimilar fashion. Also resistance to quinine seems to involve not only the PfCRT gene but also other mutations. We used flow cytometry based method to evaluate the effect of quinine on 2 chloroquine resistant (K1 and W2Mef) and 2 chloroquine sensitive (3D7 and HB3) strains of *Plasmodium falciparum*. This method allows for the stage specific study of the parasites and provides an indication of live parasites based on the accumulation of membrane potential stain. Our results showed that in a 48-hour culture, trophozoites are significantly more resistant to killing by quinine than either rings or schizonts. This resistance of the trophozoite stage, which frequently sequesters, to quinine may explain why this drug shows decreased efficacy for the rapid treatment of severe malaria.

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SYNERGISTIC INTERACTION OF THE ANTIRETROVIRAL PROTEASE INHIBITOR LOPINAVIR AND THE ANTIMALARIAL LUMEFANTRINE AGAINST *PLASMODIUM FALCIPARUM*

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New means of controlling malaria are needed. Antiretroviral protease inhibitors (PIs) have demonstrated activity against cultured malaria parasites at concentrations that are clinically relevant. For example, lopinavir acted against multiple strains of *Plasmodium falciparum in vitro* with an IC_{50} of ~1-2 µM; with standard dosing of lopinavir/ritonavir, lopinavir circulates at ~9-15 µM. We hypothesize that antiretroviral regimens containing PIs will offer protection against malaria, and we are currently comparing the incidence of malaria in Ugandan children receiving highly active antiretroviral therapy including either lopinavir/ritonavir or nonnucleoside reverse transcriptase inhibitors. To help explain potential antimalarial benefits of HIV PIs, we studied the interaction of lopinavir with various antimalarials currently used in malaria endemic areas. Lopinavir had a modest synergistic interaction with lumefantrine (fractional inhibitory interaction ± SD 0.53±0.23 for strain 3D7 and 0.66±0.32 for strain W2). Lopinavir did not show any significant interaction with chloroquine (1.42±0.62), piperaquine (1.49±0.63), monodesethylamodiaquine (1.52±0.54), dihydroartemisinin (1.34±0.57), mefloquine (1.24±0.71), or quinine (0.92±0.27). We are also selecting parasites with decreased lopinavir sensitivity to help to understand the antimalarial mode of action of this drug and potentially characterize new drug targets in malaria parasites. To date, we have selected parasites with IC_{50}s of ~4 and ~6 µM for the 3D7 and W2 strains respectively (the wild-type IC_{50} for both strains is ~2.5 µM); selection is ongoing. Our results suggest that interactions between antimalarial and antiretroviral drugs exist; including apparent modest synergy between lopinavir and lumefantrine, and should be taken into account when implementing treatment and control policies for both diseases.

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SELECTIVE REVERSAL OF PIPERAQUINE AND LUMEFANTRINE RESISTANCE IN *PLASMODIUM BERGHEI* ANKA

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Drug resistance against *Plasmodium falciparum* remains a public health problem. Strategies to overcome this problem require full understanding of the resistance mechanisms. We used *P. berghei* as a surrogate to *P. falciparum* to study antimalarial resistance. Stable lumefantrine (LM) and piperaquine (PQ) resistant *P. berghei* selected by *in vivo* drug pressure were used. To further understand the resistance mechanisms, we have tested the ability of known *P. falciparum* reversing agents, probenecid, verapamil and cyproheptadine to reverse LM and PQ resistance. Chemo-sensitization potential of probenecid, verapamil or cyproheptadine was assessed in 4-day test in which *P. berghei* in mice is exposed to four, daily drug doses. Oral treatment with LM, PQ alone or in combination with chemosensitizer was administered for a total of four daily doses. Parasite density was estimated microscopically (×100) 96 hours post parasite inoculation using thin blood films. Parent strain was sensitive to LM and PQ with an ED_{90} of 3.52 and 3.93mg/kg respectively. Lumefantrine resistant (LM^R) and piperaquine resistant (PQ^R) obtained after 1-2 years of drug pressure had ED_{90} of 52.06 and 63.39mg/kg respectively. We first tested the reversing agent alone to identify the highest doses that do not inhibit parasite growth and these doses were used to carry reversal experiments. At 5mg/kg, cyproheptadine restored LM activity by 65% against LM^R but failed to restore PQ activity against PQ^R. Probenecid (400mg/kg) and verapamil (50mg/kg) did not chemo-sensitize either LM^R to LM, or PQR to PQ. A previous study showed that PQ^R is also resistant to LM (ED_{90} 97.25mg/kg). Interestingly, these 3 chemosensitizers restored LM potency against PQ^R. In conclusion, our data shows the potential of cyproheptadine to restore LM activity in LM^R and also indicate that the selection of PQ^R is associated with LM decrease efficacy, however this efficacy can restored by chemosensitizers.

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THE ROLE OF PFMDR1 AND PFCRT IN MEFLOQUINE, LUMEFANTRINE, CHLOROQUINE AND AMODIAQUINE RESISTANCE

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Single nucleotide Polymorphisms in the PfMDR1 and PfCRT genes of *Plasmodium falciparum* may confer resistance to a number of anti-malaria drugs. PfMDR1 86Y and PfCRT 76T, have previously been linked to chloroquine resistance, with the role played by PfMDR1 being suggested as secondary compared to PfCRT. On the other hand PfMDR1 86Y is linked to mefloquine and lumefantrine sensitivity while lumefantrine has shown tolerance to parasites with PfCRT K76. We investigated the association between PfMDR86/PfCRT76 and *P. falciparum* resistance to mefloquine (MQ), lumefantrine (LU), chloroquine (CQ) and amodiaquine (AQ). *P. falciparum* field isolates were collected from malaria endemic sites in western Kenya. Genomic DNA from these isolates was genotyped

to examine mutations in PfMDR1 and PfCRT. Additionally the malaria SYBR Green I Fluorescence-based method was used to assay for *in vitro* drug sensitivity profiles (IC50), for four antimalarials. We observe that parasites lacking the PfMDR1 86Y mutation had higher mefloquine IC50s ($p<0.05$). However PfMDR1 86Y was significantly associated with higher amodiaquine IC_{50}s. While lumefantrine IC_{50}s were higher for isolates that lacked the PfCRT 76T mutation ($p <0.05$). Comparatively PfCRT 76T was observed among parasites with higher chloroquine and amodiaquine IC50s ($P<0.05$). Taken together, these results significantly link emerging MQ resistance to PfMDR1 N86. On the other hand, AQ selects for parasite with the PfMDR1 86Y and the PfCRT 76T mutations. The two mutations have been associated with chloroquine resistance and may explain the high prevalence of PfCRT 76T in Kenya in the absence of CQ pressure. Thus it is highly suggestive that the PfCRT 76T mutation will be maintained in most parasites as they respond to AQ pressure. Additionally, Parasites with PfCRT K76 are selected by LU, a partner drug in Coartem, the first-line antimalarial in Kenya. Should the parasites yield to LU pressure the result would be decreased Coartem efficacy.

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STRONGER SELECTION PRESSURE FOUND IN FLANKINGS OF SVMNT HAPLOTYPE IN COMPARISON TO CVIET HAPLOTYPE OF CHLOROQUINE RESISTANT *PLASMODIUM FALCIPARUM* ISOLATES OF INDIA

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Intensive drug pressure, confer inevitable selection of mutant parasite and its expansion in a population. The rapid spread of these favorable mutations also reduces genetic variation and increases linkage disequilibrium in the vicinity of resistance gene. Microsatellite markers flanking the upstream and downstream of *Plasmodium falciparum* chloroquine resistance transporter (*pfcrt*) *gene* have been found to be fixed among the chloroquine resistant (CQR) parasite population of Southeast Asia, South America and Papua New Guinea, having distinctive pattern of point mutations in *pfcrt gene*. To date, only a single study from central India, reported the evidence of selective sweep arround *pfcrt gene* of CQR parasite. This information is important, but raised a need of molecular surveillance throughout India for a concrete conclusion about the evolution of CQR parasite in India. Therefore, efforts were made to determine the variation among the microsatellite flanking in various *pfcrt* haplotypes found in Indian isolates, collected from 13 dispersed geographic locations representing varied *falciparum* malaria prevalence. We observed a reduced expected heterozygosity (H_e) in resistant haplotypes in comparison to the wild type (CVMNK > CVIET > SVMNT). Thus, our observation supports Wooton *et al.* theory of selective sweep around *pfcrt gene* in CQR parasite. However, stronger selection strength is observed in resistant parasite from low *P. falciparum* transmission areas as compared to high transmission areas. These observations will be valuable in understanding the evolutionary history of CQ resistant parasite in India as well as for designing the effective antimalarial drug policy.

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DRUG RESISTANT MALARIA IN CAR NICOBAR ISLAND, IN INDIA

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Drug resistance in malaria is a cause of concern for the worldwide malaria control programmes and hence forces to discover the novel antimalarial drugs. In India, the pattern of drug resistance varies from region to region. The situation of malaria in Car Nicobar Island, Andman and Nicobar Island is very different from the mainland India and the disease is transmitted by a efficient malaria vector, *Anopheles sundiacus*. Temporal rise in *pfcrt* mutations was observed previously in the parasite population in Car Nicobar Island. To support this temporal rise in *pfcrt* mutations, *in vivo* chloroquine efficacy study was undertaken to analyze the mutation in the *pfcrt* gene. A WHO protocol (1966) for assessment of therapeutic efficacy for uncomplicated *falciparum* malaria with the 28 days follow up was performed. The finger pricked blood samples spotted on the filter paper was used for the DNA isolation and then the sequencing of genes encoding for *pfcrt, pfdhfr* and *pfdhps* was done to analyze the single point mutations conferring the drug resistance. None of the isolates were observed with the wild type *pfcrt* allele and there is prevalence of triple mutant *pfcrt* allele CVIET irrespective of the chloroquine response. Majority of the patients shows treatment failure cases (60.48%, n=48) especially among the non responder (79.31%, 23 of 29). The majority of the individuals from both groups also contained parasites with the high number of two - locus PfDHFR-PfDHPS mutation associated with antifolate resistance. The results show that there is the predominance of chloroquine and antifolate resistant of *P. falciparum* malaria in Car Nicobar Island which necessitate the implementation of alternative malaria drug policy such as Artesunate Combination Therapy (ACT) for the island.

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ASYMPTOMATIC MALARIA INFECTION IN HIV-POSITIVE AND HIV-NEGATIVE NIGERIAN ADULTS

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The overlap of malaria and HIV infection in sub-Saharan Africa is a major public health issue. HIV increases the risk of *Plasmodium falciparum* infection progressing to clinical malaria in adults, especially in those with advanced immunosuppression, by eroding acquired immunity. Increasing parasite burdens and reduced host immunity, both of which occur with HIV infection, are associated with increased treatment failure rates. The study was designed to address the paucity of data regarding the use of Artemether-lumefantrine (AL) among HIV-positive subjects in Nigeria despite the high prevalence of asymptomatic malaria infection in Nigerian adults. Field work was carried out in Port Harcourt, Nigeria which is rich in the nation's oil resources. The region is dotted with oil and gas activities, and commercial sex workers follow the camp. The resultant effect is a high prevalence of HIV. Participants aged between 16-65yrs were recruited from the HIV adult and general OPD clinics of the University of Port Harcourt Teaching Hospital, Braithwaite Memorial Hospital and also among students of the University. Finger prick and venous blood samples were collected as blood spots on filter papers and in EDTA tubes. DNA extracted from blood spots will be used for detection of malarial parasites by PCR, and carriage of drug resistance markers on parasite-positive samples before and after treatment in both HIV-positive and HIV-negative participants. Blood levels of antimalarial drugs will also be measured in both groups. This pilot study in HIV-positive patients will assess the prevalence of resistance markers to anti-folates used for prophylaxis against opportunistic infections among this vulnerable group of people, and provide an estimate of the efficacy of AL in this setting. Pharmacokinetic analyses will provide preliminary

information as to whether the current AL dosing regimen is sufficient for HIV-positive patients. The study will provide initial data to inform future larger studies, and will therefore help to inform policy in the treatment of malaria in HIV subjects.

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ARE MOZAMBICAN MALARIA PARASITES CHLOROQUINE SENSITIVE OR LUMEFANTRINE RESISTANT?

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Over the past five year, the first line malaria treatment in Mozambique has changed twice. In 2006 the artemisinin based combination, artesunate plus sulfadoxine-pyrimethamine replace chloroquine, which in turn was replaced by artemether-lumefantrine in 2008. This study aimed to assess the effect these drug policy changes had on the prevalence of molecular markers associated with chloroquine and lumefantrine resistance in Gaza Province, Mozambique. Community based asexual parasite prevalence surveys were conducted annually at 38 sentinel site in Gaza Province from 2006 to 2010. Finger prick blood spots were collected from RDT malaria positive children (aged between 2 and 15) at each site. Parasite DNA extracted from the blood spots was subjected SNP analysis to detect mutations at *crt*76 and *mdr*86 codons. The copy number of the *mdr1* gene was also assessed. At baseline both the *crt*76T and *mdr*86Y mutations were approaching saturation within the population, with prevalences of 96.1% and 74.7%, respectively. Following the replacement of chloroquine with combination therapy, prevalence of these two markers began decreasing. By 2010 the prevalence of the *crt*76T mutation was 32.4% while the *mdr*86Y mutation prevalence was 30.9%. As chloroquine drug pressure decreased in the region, so did the mutation markers associated with cholorquine resistance. Although the artemether-lumefantrine became national policy in 2008, the complete roll out of this drug to all health facilities across Gaza Province took at least two years to be achieved. Therefore the increase in prevalence of the mdr86N allele is more likely a result of decreased chloroquine pressure rather than increased lumefantrine pressure. However close surveillance of the *mdr*86N prevalence as well as *mdr1* copy number is needed as lumefantrine drug pressure increases within the region.

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STUDYING *PLASMODIUM FALCIPARUM* GENOTYPE AND PHENOTYPE TO ASSESS THE RELIABILITY OF DAPI-BASED *EX VIVO* ASSAY FOR MONITORING PARASITOLOGICAL RESPONSES TO ANTI-MALARIAL DRUGS IN SENEGAL

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Malaria remains an important public health issue in developing countries, despite efforts to reduce morbidity and mortality from this disease. The spread of *Plasmodium falciparum* drug resistance is outpacing new anti-malarial development and compromising effective malaria treatment. To maximize utility of available drugs, periodic monitoring of drug efficacy and gathering of accurate information regarding changes in parasite sensitivity are essential. We have recently developed a non-radioactive field-based DAPI assay to evaluate *ex vivo* anti-malarial drug sensitivity of *P. falciparum,* as reported previously. In this study we assessed the correlation between the *ex vivo* drug profile of field isolates and known drug resistance markers using High Resolution Melting (HRM) technology. Blood samples were collected from patients with clinical malaria during the three-month (September to December) transmission season in years 2008 and 2009 in Thies, Senegal. Blood samples containing 0.2 - 1% parasitemia were incubated with various drugs to determine IC_{50} values. A number of these samples containing single-genome infections were then culture adapted, and the drug assay repeated *in vitro*. We observed good correspondence between the *ex vivo* and *in vitro* drug IC50 values, demonstrating that the *ex vivo* assay provides reliable results regarding drug phenotype. Analysis of this phenotype data and the genotype data from the HRM assays demonstrated a significant association between Pfmdr1N86Y and Pfmdr1Y184F alleles and response to mefloquine; Pfdhfr S108N and response to pyrimethamine; and Pfcrt K76N and response to chloroquine. These results show that the non radioactive *ex vivo* DAPI based drug assay is reliable and can be used to assess parasitological responses to anti-malarial drugs in the field. This assay may be used in the field to serve as an early warning system to detect decreased parasite responses to antimalarial drugs before clinical failures are evident.

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PREVALENCE, DISTRIBUTION AND ORIGIN OF DRUG RESISTANT *PLASMODIUM* PARASITES IN THE SOUTH PACIFIC ISLANDS OF VANUATU AND THE SOLOMON ISLANDS

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Limited data exists on drug resistant malaria in the Solomon Islands (SI) and Vanuatu. Using samples collected from baseline epidemiological surveys in Tafea Province, Vanuatu and Temotu and Malaita Provinces, SI we investigated the prevalence, distribution and origin of drug resistant *Plasmodium* parasites by examining sequence polymorphisms within pfcrt, pfdhfr, pfdhps, pvdhfr and pvdhps. Analysis of the Pfcrt gene revealed 100% (Tafea and Malaita) and 98% (Temotu) of parasites carried the K76T polymorphism. The dominant mutant Pfcrt allele observed in Vanuatu and SI is similar to that found in Papua New Guinea (PNG). Analysis of microsatellite (MS) markers flanking pfcrt, combined with Pfcrt fingerprints, provides indications on the origin of drug resistance in these provinces. In SI and Vanuatu, 74% and 66% of the Pfcrt mutant alleles exhibited identical size in 4 of the 5 MS markers compared to those flanking mutant PNG Pfcrt allelic types; suggesting that these CQR parasites share a common ancestry. In Vanuatu three distinct *pvdhfr* alleles were observed with the majority of isolates containing the double polymorphism, 58R /117T. A novel substitution at residue 57 where a methionine residue replaced the wildtype phenylalanine occurred in 21% of the samples. Similarly, genotyping of *Pfdhfr* revealed a dominance of the double polymorphism 59R/108N. In Malaita the most common *pvdhfr* allele was the quad mutant 57L/58R/61M/117T. A novel mutation at aa 173 was identified in one sample where isoleucine was replaced by a methionine. Unlike the variability exhibited in the *pvdhfr* gene, 100% of samples possessed a drug sensitive *pvdhps* allele for which the most common allelic type identified was 382S/383A/512K/553A/585V. Surveillance is vital for the employment of effective drug treatments. Understanding drug sensitivity patterns may assist malaria eradication efforts currently underway in the South Pacific.

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MODULATING EFFECTS OF PLASMA CONTAINING ANTI-MALARIAL ANTIBODIES ON *IN VITRO* ANTI-MALARIAL DRUG SUSCEPTIBILITY IN *PLASMODIUM FALCIPARUM*

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The efficacy of anti-malarial drugs is determined by the level of parasite susceptibility, anti-malarial drug bioavailability and pharmacokinetics, and host factors including immunity. Host immunity improves the *in vivo* therapeutic efficacy of anti-malarial drugs, but the mechanism and magnitude of this effect has not been characterized. This study characterized the effects of 'immune' plasma to *Plasmodium falciparum* on the *in vitro* susceptibility of *P. falciparum* to anti-malarial drugs. Titres of antibodies against blood stage antigens (mainly the ring-infected erythrocyte surface antigen [RESA]) were measured in plasma samples obtained from Thai patients with acute *falciparum* malaria. 'Immune' plasma was selected and its effects on *in vitro* parasite growth and multiplication of the Thai *P. falciparum* laboratory strain TM267 were assessed by light microscopy. The *in vitro* susceptibility to quinine and artesunate was then determined in the presence and absence of 'immune' plasma using the ^{3}H-hypoxanthine uptake inhibition method. Drug susceptibility was expressed as the concentrations causing 50% and 90% inhibition (IC_{50} and IC_{90}), of ^{3}H-hypoxanthine uptake. Incubation with 'immune' plasma reduced parasite maturation and decreased parasite multiplication in a dose dependent manner. ^{3}H-hypoxanthine incorporation after incubation with 'immune' plasma was decreased significantly compared to controls (median [range]; 181.5 [0 to 3,269] cpm versus 1,222.5 [388 to 5,932] cpm) ($p = 0.001$). As a result 'immune' plasma reduced apparent susceptibility to quinine substantially; median (range) IC_{50} 6.4 (0.5 to 23.8) ng/ml versus 221.5 (174.4 to 250.4) ng/ml ($p = 0.02$), and also had a borderline effect on artesunate susceptibility; IC_{50} 0.2 (0.02 to 0.3) ng/ml versus 0.8 (0.2 to 2.3) ng/ml ($p = 0.08$). Effects were greatest at low concentrations, changing the shape of the concentration-effect relationship. IC_{90} values were not significantly affected; median (range) IC_{90} 448.0 (65 to > 500) ng/ml versus 368.8 (261 to 501) ng/ml for quinine ($p > 0.05$) and 17.0 (0.1 to 29.5) ng/ml versus 7.6 (2.3 to 19.5) ng/ml for artesunate ($p = 0.4$). 'Immune' plasma containing anti-malarial antibodies inhibits parasite development and multiplication and increases apparent *in vitro* anti-malarial drug susceptibility of *P. falciparum*. The IC_{90} was much less affected than the IC_{50} measurement.

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ASSOCIATION OF GENES POLYMORPHISMS IN THE SUSCEPTIBILITY TO MALARIA IN THREE ETHNIC GROUPS LIVING IN STABLE AND SEASONAL MALARIA TRANSMISSION AREA OF BURKINA FASO

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Previous studies show that human genetic variation can affect malaria susceptibility. Previous genetic studies investigating the responses in human malaria show consistent differences in *Plasmodium falciparum* parameters between Mossi and Fulani; the latter are clearly less parasitized, and less affected by the disease. This raises the possibility that quantitative analysis within Fulani may be an efficient way of screening for important genetic factors. This study was undertaken to assess the role of genetic polymorphism in the susceptibility to malaria in three ethnic groups in Burkina Faso. We performed a total of five cross sectional and two longitudinal surveys during 2007 and 2008 in four rural villages near Ouagadougou. For each subject, clinical data was collected, antibodies (Abs) against specific malaria antigens measured by ELISA and 170 malaria associated SNPs genotyped. *P. falciparum* infection rates and clinical malaria incidence were lower in Fulani (P=0.001) compared to Mossi and Rimaibe groups. Our results showed that the titers of Abs generated against all antigens tested were significantly (P=0.005) higher in Fulani compared to the sympatric group. Logistic regression analysis with antibody, gender, and age-group as covariates identified significances for SNPs in IL13,INFG,IL7R,IL22,TNF,IL1B,IL10 in the Fulani, in CSF2,IL3,IL22,IL1B in the Rimaibe and in IL3,IL13,IL4,IL10 in the Mossi. In conclusion, the present study revealed associations between host genetic factors and either the clinical, parasitological or immunological status for different ethnic groups with *P. falciparum* malaria. Although many SNPs were significantly associated with clinical malaria and high antibody titers, further work particularly in Fulani is warranted to understand function.

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FCΓRIIA POLYMORPHISM AND ANTI-MALARIA SPECIFIC IGG AND IGG SUBCLASSES IN POPULATIONS WITH DIFFERENT SUSCEPTIBILITY TO MALARIA IN BURKINA FASO

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On human leucocytes there are three distinct classes of IgG receptors (FcγR) currently recognized, FcγRI, FcγRII and FcγRIII. FcγRIIa is known to be polymorphic; this functional polymorphism is associated with differing susceptibilities to malaria. Several studies reported that Fulani are less affected by clinical malaria than non-Fulani groups despite similar exposure and an ethnic difference in FcγRIIa genotype frequencies. No previous studies have addressed these questions in Burkina Faso. The present study aims to assess difference in susceptibility to malaria between Mossi and Fulani, and influence of FcγRIIa polymorphism on anti- *falciparum* malaria antibody responses Healthy adults more than 20 years from Mossi and Fulani were enrolled for the assessment of immunological and genetic endpoints in relation with susceptibility to malaria during malaria transmission season. A clinical examination was performed to check medical history of study participants. Urine was requested from women to check their pregnancy status. 20 ml of venous blood were collected into heparin tubes. DNA was extracted from collected blood samples and FcγRIIa polymorphism was investigated. Antibody levels against *Plasmodium falciparum* antigens (MSP3, MSP2b, MSP2b, GST, Pf10 and GLURP-R0) were measured by ELISA. When comparing parasite density, we observed that it was relatively lower in the Fulani group than the Mossi group, (p = 0.01). Regarding antibody levels, we found, that the Fulani had higher antibody levels than the Mossi group indepently of the malaria transmission season. In both ethnic groups a similar distribution of homozygotes carrying the 131 R/R and the 131H/H as well as heterozygotes for the 131H/R was found. When comparing the allele frequencies, the R allele was dominant in both ethnic groups compared to H allele. Regarding FcγRIIa polymorphism on *P. falciparum*-reactive antibody levels, we found that in each genotype group, the Fulani had higher antibody levels than the Mossi group. In conclusion, this study shows that Fulani are less affected by clinical malaria than non-Fulani group. Contrary to all expectations, this study show none ethnic difference in FcγRIIa genotype frequencies between the Fulani and non-Fulani groups. Rather correlation analysis between antibody levels and FcγRIIa R131H polymorphisms revealed that the Fulani had higher antibodies than Mossi for all genotype groups.

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CD47 EXPRESSION ON ERYTHROCYTE OF CHILDREN WITH SEVERE *PLASMODIUM FALCIPARUM* MALARIA

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CD47 (integrin associated protein), is expressed on numerous cell types, including RBCs and protects against phagocytosis via macrophages by binding to the inhibitory macrophage receptor SIRPα. We hypothesized that high CD47 expression would protect against severe malaria (SA) due to acute malaria, which is partly caused by erythrophagocytosis. We measured CD47 expression on RBC by means of flow cytometry in patients with severe malaria, (severe malaria anaemia, SA, n=11; malaria with intravascular haemolysis, IVH, n=8; cerebral malaria, CM, n=9), uncomplicated malaria (UM, n=10) and asymptomatic control (AC, n=9). CD47 expression was similar on RBCs from patients with SA+IVH (29.6, 95%CI (26.7-32.4) when compared with CM (28.9, 95%CI (23.9-33.9), UM (26.2, 95%CI (23.2-29.2) and control (26.4, 95%CI (23.9-28.8), p=0.27. The surprising result could imply a limited role for immune-mediated erythrophagocytosis but needs to be confirmed in longitudinal studies.

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MICRO-GEOGRAPHIC HETEROGENEITIES IN EXPOSURE TO *ANOPHELES GAMBIAE* SALIVARY GLAND PROTEIN IN DIFFERENT MALARIA ENDEMICITIES IN THE WESTERN KENYA HIGHLANDS

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Improvement in malaria control in low transmission settings, such as western Kenya, requires sensitive and reliable tools to facilitate current malaria risk evaluation programs. Quantification of human antibody responses to *Anopheles gambiae* salivary gland protein (gSG6-P1) as immuno-epidemiological marker of exposure to *Anopheles* bites in an exposed population has shown promise. This study measured total IgG responses to gSG6-P1 protein in an age stratified cohort (<5, 5-14, ≥15, n=800) from Kakamega, western Kenya. The purpose was to evaluate the micro-heterogeneities in human exposure to *Anopheles* vectors in different malaria endemic localities at different altitudes and malaria transmission intensities and how this correlate with risk of parasite transmission. In addition, we examine how our previous finding -"marked heterogeneities in IgG responses to Pf MSP119 in an uphill and valley bottom residents" (n =800, Kakamega), that shows exposure to parasite, correlate with gSG6-P1 which reflect exposure to the vector. Serum samples were obtained in Mar-Apr. and Jun-July 2009. Additional samples (n=635) were received from a high-transmission 'lowland' area (Kombewa, EIR 31) and a low-transmission highland area (Kisii, EIR 0.04) and Kakamega from Jan-Mar 2011. Parasite prevalence was determined by both standard microscopy and HRP2 based RDT. Preliminary results (n=1305) showed that in Kakamega, there was a significant difference between median ODs (Mann-Whitney test) from uphill and the valley residents (p= 0.0023). Analysis of age stratified responses also revealed significant differences (p=0.0058), with the younger age groups differing with the ≥15 age group. IgG responses to gSG6-P1 differed significantly between Kombewa (230), Kakamega (n=200) and Kisii (205) as would be expected for parasite rates of 65%, 19.2% and 3% respectivelyand correlates with MSP119 responses. These results confirm gSG6-P1 as a highly sensitive and a robust marker of exposure to vector bites and parasite exposure even in low transmission settings such as the highlands of western Kenya.

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DIFFERENTIAL ACQUISITION OF HUMAN ANTIBODY RESPONSES AGAINST *PLASMODIUM FALCIPARUM* ACCORDING TO THE INTENSITY OF EXPOSURE TO *ANOPHELES* BITES

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Immunity to *Plasmodium falciparum* malaria is modulated by many environmental and epidemiological factors. This study evaluates the influence of the impact of human exposure to *Anopheles* bites, on the acquisition of antibody responses to *P. falciparum* in children living in malaria area.120 children aged from one to nine years were selected in two Senegalese villages (Gankette vs. Mboula) where the intensity of exposure to *Anopheles* bites was markedly different (high vs. low exposure respectively). In this cohort, specific IgG, IgG1 and IgG3 responses to *P. falciparum* whole schizont extract (WSE) and circumsporozoite protein (CSP) were evaluated before (June) at the peak of *Anopheles* exposure (September) and later (December). Multivariate analysis showed a significant decrease in IgG and IgG1 against *P. falciparum* WSE and CSP in children highly exposed to *Anopheles* bites compared to those who were weakly exposed. This difference between both villages remained similar when considering only uninfected children. In contrast, in both villages, parasitemia and increasing age were strongly associated with higher IgG, IgG1 and IgG3 levels. High exposure to *Anopheles* bites appeared to down-modulate IgG and IgG1-dependent responsiveness to *P. falciparum* known to induce protective immune responses against malaria infection. Further research will add to our understanding of the immunological consequences of mosquito saliva on the complexity of the interactions between the malaria parasite and its host.

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MALARIA INFECTED INDIVIDUALS IN THE ENDEMIC AREA CARRY ANTIBODIES TO *PLASMODIUM FALCIPARUM* MATURE GAMETOCYTES

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In a recent study in The Gambia, mature gametocyte-infected erythrocytes of *Plasmodium falciparum* were found to carry antigens (gametocyte surface antigens, GSA) that were recognized by malaria patient's plasma antibodies. These were called anti-GSA antibodies and were associated

with lower duration of gametocyte carriage in these patients3,4. Thus, we would like to determine epidemiological patterns in GSA antibody prevalence and density and seek evidence for specific immune suppression of gametocytes induced by GSA antibodies.To confirm the concept of GSA and to determine its relevance for *P. falciparum* gametocyte carriage in natural populations, we conducted an immuno-epidemiology study in asymptomatic school children in a rainforest region in Ghana. In this study we enrolled parasite positive children who did not show any clinical symptoms of malaria, treated them with dihydro-artemisinin piperaquine to clear asexual parasitaemia and followed them up weekly for one month, each time with finger-prick blood collection for plasma and RNA. The RNA samples were for gametocyte detection by NASBA and the plasma for gametocyte antibody detection by flow cytometry. So far, whereas 8.9% (15/168) of the children enrolled were found to be gametocyte carriers at enrolment, 32 (19%) of them developed gametocytes during the follow up period by microscopy. We anticipate higher gametocyte prevalence, perhaps twice or more by NASBA detection. We however expect a GSA antibody prevalence of 34% (as found in the Gambian data) in the sub-group of children with gametocytes at enrolment, and 10% or more in the sub-group that developed gametocytes during the follow-up, a proportion that increases after gametocytes have been developed. We also expect ≤10% of the sub-group of children who remain gametocyte free throughout the study. Further flow cytometry and NASBA experiments are ongoing so we can answer the questions: are gametocyte antibodies related to short clearance time of gametocytes in the presence or absence of treatment and how rapidly are these developed.

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INFLUENCE OF INTERMITTENT PREVENTIVE TREATMENT USING SULFADOXINE - PYRIMETHAMINE ON ANTIBODY RESPONSES TO *PLASMODIUM FALCIPARUM* IN PREGNANT WOMEN IN CAMEROON

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Antibodies (Ab) towards malaria antigens are important in protection against clinical malaria. Immunity to malaria is known to be short lived with Ab levels dropping when individuals are not exposed to malaria. The World Health Organization recommends that pregnant women in malaria endemic countries receive Intermittent Preventive Treatment (IPT) using sulphadoxine and pyrimethamine to prevent the severe effects of malaria, including maternal anemia and low birth-weight babies. It is unclear, however, if the absence of boosting during pregnancy will result in lower Ab levels that mediate protection. Accordingly, we investigated if IPT-SP had an influence on humoral immune responses to 9 malaria antigens in pregnant women living in a rural village in Cameroon. Pregnant women (n=76) were recruited at their first prenatal visit and followed monthly until delivery. Blood samples were collected monthly and each woman received an average of 2.7 doses of SP. IgG Ab levels were determined using a multiplex-analyte bead-based assay and results were recorded as median fluorescent intensity. The antigens included 8 antigens that are important in immunity against malaria in all individuals and to one antigen, VAR2CSA, that is important only during pregnancy as it helps present infected erythrocytes from sequestering in the placenta. For comparison, samples from a longitudinal study conducted in the same village before the use of IPT-SP were studied (n=37 women). Results showed that at delivery women who received IPT throughout pregnancy did not have lower levels of IgG to the 8 asexual stage-antigens compared to the control group, but their IgG levels to VAR2CSA were reduced ($p = < 0.0001$). Since antibodies towards VAR2CSA are important for protection against placental malaria, it is recommended that primigravidae who received IPT use other protective methods for preventing malaria in subsequent pregnancies, as they may not produce protective Ab while taking IPT.

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THE INFLUENCE OF INTERMITTENT PREVENTIVE TREATMENT USING SULFADOXINE-PYRIMETHAMINE ON THE REPERTOIRE OF ANTIBODIES TO DIFFERENT DOMAINS AND VARIANTS OF VAR2CSA IN PREGNANT WOMEN IN CENTRAL AFRICA

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VAR2CSA is a member of the *Plasmodium falciparum* erythrocyte membrane protein-1 family of adhesion molecules. It is a transmembrane-protein consisting of six Duffy-Binding-Like (DBL 1-6) domains that are expressed on the surface of infected RBC sequestered in the placenta of pregnant women. Antibodies against VAR2CSA improve pregnancy outcomes. Thus, information on which VAR2CSA domains are important in protection is crucial for development of a vaccine to protect pregnant women. In the last 6 years, the World Health Organization introduced intermittent preventive treatment (IPT) using sulfadoxine-pyrimethamine and insecticide-treated bed-nets to prevent malaria during pregnancy. The goal of this study was to investigate if women on IPT treatment acquire VAR2CSA antibodies (Ab), and if so, to which domains. Between 2009 and 2010, 68 women living in Ngali II, Cameroon, who received IPT were followed throughout pregnancy and their Ab levels against 5 DBL domains from 3 parasite strains (3D7, 7G8, and FCR3) were measured using a multiple-analyte bead-based assay. Similar samples from 39 women collected before the initiation of IPT were evaluated for comparison. Results showed that women who started taking IPT during the first trimester produced less Ab to DBL domains 3, 5 and full length VAR2CSA. As expected, women who started receiving IPT during the second and third trimesters produced higher levels of Ab to all VAR2CSA domains compared to women enrolled during the first trimester. Finally, multigravidae women on IPT, who enrolled late in the pregnancy, had Ab to more VAR2CSA domains compared to multigravidae women who enrolled early in pregnancy. The results suggest that women start to make anti-VAR2CSA antibodies early in pregnancy, but Ab responses continue to expand as pregnancies progress. The data show that IPT treatment reduced both the amount and repertoire of VAR2CSA Ab in pregnant women living in a high malaria transmission area. Measuring Ab towards VAR2CSA domains may be used to evaluate the efficacy/compliance to IPT.

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THE INFLUENCE OF INTERMITTENT PREVENTIVE TREATMENT USING SULFADOXINE-PYRIMETHAMINE ON THE REPERTOIRE OF ANTIBODIES TO DIFFERENT DOMAINS AND VARIANTS OF VAR2CSA IN PREGNANT WOMEN IN CENTRAL AFRICA

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VAR2CSA is a member of the *Plasmodium falciparum* erythrocyte membrane protein-1 family of adhesion molecules. It is a transmembrane-protein consisting of six Duffy-Binding-Like (DBL 1-6) domains that are expressed on the surface of infected RBC sequestered in the placenta of pregnant women. Antibodies against VAR2CSA improve pregnancy outcomes. Thus, information on which VAR2CSA domains are important in protection is crucial for development of a vaccine to protect pregnant women. In the last 6 years, the World Health Organization introduced intermittent preventive treatment (IPT) using sulfadoxine-pyrimethamine and insecticide-treated bed-nets to prevent malaria during pregnancy. The goal of this study was to investigate if women on IPT treatment acquire VAR2CSA antibodies (Ab), and if so, to which domains. Between 2009 and 2010, 68 women living in Ngali II, Cameroon, who received IPT were followed throughout pregnancy and their Ab levels against 5 DBL domains from 3 parasite strains (3D7, 7G8, and FCR3) were measured using a multiple-analyte bead-based assay. Similar samples from 39 women collected before the initiation of IPT were evaluated for comparison. Results showed that women who started taking IPT during the first trimester produced less Ab to DBL domains 3, 5 and full length VAR2CSA. As expected, women who started receiving IPT during the second and third trimesters produced higher levels of Ab to all VAR2CSA domains compared to women enrolled during the first trimester. Finally, multigravidae women on IPT, who enrolled late in the pregnancy, had Ab to more VAR2CSA domains compared to multigravidae women who enrolled early in pregnancy. The results suggest that women start to make anti-VAR2CSA antibodies early in pregnancy, but Ab responses continue to expand as pregnancies progress. The data show that IPT treatment reduced both the amount and repertoire of VAR2CSA Ab in pregnant women living in a high malaria transmission area. Measuring Ab towards VAR2CSA domains may be used to evaluate the efficacy/compliance to IPT.

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REGULATORY T CELLS ARE NOT IMPLIED ON REGULATION OF *PLASMODIUM FALCIPARUM* INFECTION IN INDIVIDUALS LIVING IN PERUVIAN AMAZON

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Several studies have demonstrated that regulatory T cells (Tregs) play a critical role balancing protective immune responses and mediate pathology during malaria infection. These cells suppress cellular immune responses through direct contact with immune effector cells by producing regulatory cytokines as TGF-ß and IL-10, suggesting that Tregs may contribute to the onset of *P. falciparum* infection. The objective of the study was to find the association of Tregs with the clinical outcome of individuals infected with *P. falciparum* living in a hypoendemic malaria region. CD4+CD25+CD127loFoxp3+ Tregs were identified by flow cytometry and reported as percentage of total CD4+ T cells in three groups: symptomatic (S), asymptomatic (AS) and control (C) individuals. PBMCs from each individual were cultured using the recombinant C-terminal repeat region GLURP-R2 antigen. Concentrations of IL-10, TNF-α and IFN-γ from the culture supernatant were measured each day during 6 days. This study showed that S, AS and C groups presented similar Tregs percentage (3.89%, 3.47% and 3.51% respectively) in peripheral blood. Furthermore, there was no a positive correlation between parasitemia and Tregs percentage (P-Value= 0.47). TNF-α levels were the highest in PBMCs cultures in S group (>1440.24 pg/ml), IL-10 stayed low (~200 pg/ml) over the first four days of culture, having a peak during the 6th day (759.28 pg/ml). IFN-γ levels were low (347.6 pg/ml). In AS group, TNF-α had a discreet high level at the 1st day (697.6 pg/ml) and going down (~130 pg/ml) during the next 5 days. IL-10 stayed state (around 363.55 pg/ml) during the all days. It was also observed very low concentrations of IFN-γ against GLURP-R2 during the first six days. All groups (S, AS, and C) presented similar Tregs percentage and there was no positive correlation with parasitemia levels, suggesting that Tregs may are not implicated in the control and/or exacerbation of parasite multiplication. Thus, it seems imply a control by Th1 and Th2 response instead of Tregs control during malaria infection in this population.

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ANTIBODIES TO *PLASMODIUM FALCIPARUM* ERYTHROCYTE BINDING ANTIGEN-175 AND PROTECTION FROM CLINICAL MALARIA

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Antibodies to blood-stage *Plasmodium falciparum* antigens have been associated with protection against clinical malaria in some studies but not others. Many of these studies have not assessed whether high-level antibodies are associated with protection and have not adjusted for differences in malaria exposure. The presence of high-level antibodies to apical membrane antigen-1 (AMA-1), erythrocyte binding antigen-175 (EBA-175) and merozoite surface protein-119 (MSP-119) was assessed in eighty-seven children living in a malaria holoendemic area of Kenya. The children were prospectively assessed over one year for clinical malaria. In unadjusted analyses, high-level antibodies to MSP-119, but not EBA-175 or AMA-1, were associated with protection from clinical malaria. However, after adjustment for exposure, only high-level antibodies to EBA-175 were associated with protection from clinical malaria (hazard ratio (HR), 0.48, 95% confidence interval (CI) 0.24, 0.95, P=0.03), and with reduced episodes of clinical malaria (incidence rate ratio, 0.50, 95 % CI, 0.31, 0.81, P=0.005). A trend toward increased protection from clinical malaria in children was seen with antibodies to both EBA-175 and MSP-119 (HR, 0.26, 95% CI 0.03, 1.94, P=0.18) High-level antibodies to EBA-175 are associated with protection from clinical malaria in children in a malaria holoendemic area of Kenya. Accurate estimates of antibody-associated protection from clinical malaria require adjustment for malaria exposure.

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HEME MEDIATED STAT3 ACTIVATION IN SEVERE MALARIA

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Severe forms of *Plasmodium falciparum* malaria including cerebral malaria (CM) and severe malaria anemia (SMA) are often complicated

by associated acute lung injury (ALI), acute respiratory distress syndrome (ARDS) and acute renal failure which increase the risk of fatal disease. Heme oxygenase (HO) is the rate-limiting step enzyme that breaks down Heme to biliverdin, carbon monoxide (CO) and iron. Increased HO-1 provides protection against Heme-related cellular stress. Since HO-1, CXCL10/CXCR3 and signal transducer and activator of transcription (STAT3) have been shown to be activated by pro-inflammatory stimuli and cellular stresses, we hypothesized that STAT3 might mediate the signaling pathways in severe malaria. Using a murine model of experimental cerebral malaria (ECM), we demonstrated that infection of C57BL/6 with *P.berghei* ANKA causes multiple organ damage including disruption of blood vessel walls by endothelial apoptosis and degeneration, edema, parenchymal microhaemorrhages, vascular plugging and necrosis in various tissues. Infection of C57BL/6 mice with *P. berghei* up-regulated HO-1 in several tissues, suggesting HO-1 expression may be protective against *P. berghei* induced damage. CXCL10-/- mice downregulated HO-1, suggesting that transcription of mouse *HO-1* gene is positively regulated by CXCL10. Interestingly, upregulated pSTAT3 protein was observed in various tissues of C57BL/6 mice infected with *P. berghei*. However, *P. berghei* infection failed to upregulate HO-1 protein in CXCL10-/- mice. Consistent with increased production of HO-1 detected during malaria infection in ECM mice, free Heme levels increased in WT but relative low when CXCL10 was deficient. Fatal ECM is associated with increased expression of *CXCL10* in vital organs in C57BL/6 WT mice. In *in vitro* studies, expression of HO-1 and CXCL10 were significantly up-regulated by Heme and its inducer and down-regulated by its inhibitor. Furthermore, CXCL10 was activated by Heme at the transcriptional level. pSTAT3 was consistently induced by Heme. siSTAT3 or its pharmacological inhibitor, AG-490 inhibited HO-1 expression induced by Heme. Taken together, our results indicate that the Heme/HO-1 and CXCL10/CXCR3 systems play important roles in the pathogenesis of severe forms of malaria and that STAT3 might be a critical mediator of signaling pathways involved in severe malaria pathogenesis.

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MSP-1 AND MSP-2 ALLELE SIZES, PEAK HEIGHT AND PEAK AREA AS GENETIC MARKERS FOR STUDYING *PLASMODIUM FALCIPARUM* POPULATION STRUCTURE

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Plasmodium falciparum population structures in endemic areas are characterized by extensive genotype diversity. In this study we evaluated electropherograms from high resolution capillary electrophoresis to determine if allele diversity and density for MSP-1 and MSP-2 allelic families as defined by allele size, peaks height and area can provide reproducible criteria for studying *P. falciparum* population structure. DNA samples for parasite genotyping came from a case control study that enrolled 120 children with either severe malaria anemia or uncomplicated malaria. Replicate primary amplifications were followed by nested PCR using fluorescently labeled primers targeting MSP-1 (K1, MAD20 and RO33) and MSP-2 (FC27 and IC3D7) allelic families. Following capillary electrophoresis, the electropherograms were evaluated for variations in allele size, peak height and peak. In replicate assays, the allele numbers and sizes (up to 1 nucleotide difference) were reproducible in every case. Relative abundance of the alleles as given by peak heights were more reproducible (highest % standard deviation = 5.5) than for peak area (highest % standard deviation = 46.3%). This study has demonstrated that allelic sizes and numbers from a high resolution capillary electrophoresis are reproducible and that, allele peak height is preferable to peak area in defining relative abundance of alleles. The use of allele peak adds a third dimension for quantifying *P. falciparum* clone density.

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PATTERNS OF ANTIGEN VARIATION IN ASYMPTOMATIC, UNCOMPLICATED AND SEVERE *PLASMODIUM FALCIPARUM* MALARIA IN INDIA

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The study of the nature and extent of *Plasmodium falciparum* genetic diversity is important for understanding the role the parasite may play in imparting different outcomes of malaria infection. Previous studies in various geographical regions of the malarious world have shown differences in parasite genotype distribution between different clinical groups. In our project in India, we have analyzed the association between the diversity of the parasite and its influence on three different malaria disease outcomes (asymptomatic, uncomplicated and severe) in isolates collected from Orissa, a hyperendemic area for *P. falciparum*, in high transmission season during 2008-2009. We have genotyped ~40 samples from asymptomatic patients, ~70 samples from uncomplicated patients, and ~50 from severe patients, for Merozoite Surface Protein-1 (*MSP-1*), Merozoite Surface Protein-2 (*MSP-2*), Knob-Associated Histidine Rich Protein (*KAHRP*), and Erythrocyte Binding Antigen-175 (*EBA-175*). *MSP-1* and *MSP-2* showed extensive genetic diversity among the isolates, and allele frequency distribution showed differences in the distribution of alleles among the three different malaria outcomes. Genotype distribution of *KAHRP* and *EBA-175* dimorphism also revealed polymorphism among the isolates, but no significant difference was observed between asymptomatic, uncomplicated and severe patients. Our study on the characterization of parasite genotypes with disease outcome reveals that isolates from Orissa are highly genetically diverse and that the parasite genotypes may play a role in imparting different outcomes of malaria.

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DIFFERENCE IN *PLASMODIUM FALCIPARUM* EBA-175, AMA-1 AND MSP-3 HAPLOTYPES FROM CHILDREN UNDER FIVE YEARS WITH ASYMPTOMATIC OR SYMPTOMATIC MALARIA LIVING IN AN HIGH MALARIA TRANSMISSION AND MARKEDLY SEASONAL SETTING OF BURKINA FASO

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Merozoite surface antigens are generating immunological responses associated to protection against clinical malaria and are currently under development as candidate malaria vaccine. EBA-175, AMA1 and MSP3 present some extent of variation across the entire sequences of the coding respective genes. Therefore, *eba-175 eba-175*, *ama*-1 and *msp3* gene analysis from natural *P. falciparum* populations could contribute to the design of malaria vaccine development in malaria seasonal an endemic settings and to well understand malaria pathogeny.The main purpose is to investigate whether the *eba-175*, *ama*-1 and *msp3* haplotypes were different from symptomatic and asymptomatic malaria children under five years living in Burkina Faso. Blood filter papers were collected during 2008 malaria transmission season from 289 and 231asymptomatic and symptomatic children under five years of age, respectively, living in rural

area of Saponé at about 50 km from Ouagadougou, the capital city. Parasite DNA was extracted by QIAGEN Kits and the haplotypes diversity assessed by a nested PCR followed by digestion (enzyme restriction) based on the polymorphism region of the *eba-175, ama-1 and msp3* genes. The prevalence of *eba_FCR3*, haplotypes were significant (p<0.0001) high in asymptomatic children (80.1%) compared to symptomatic children (61.5%). In contrast the *eba-175_CAMP* (41.9%), *msp3_K1* (59.4%) haplotypes were statistically more prevalent in symptomatic compared to asymptomatic children (P<0.0001). However, no difference was observed in the prevalence of *msp3_3D7* haplotypes (p=0.1) as well as in the distribution of *ama1*_haplotypes (3D7, p=0.2; K1, p= 0.5; and HB3, p= 0.6). These results showed that the *eba-175* and *msp3 Plasmodium falciparum* haplotypes may play a role in malaria pathogenicity. This information can also be used for designing malaria clinical trial using vaccine formulations based on these antigens.

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PROTEIN TARGETING PARASITOPHOROUS VACUOLE MEMBRANE OF *PLASMODUIM FALCIPARUM*

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Parasitophorous vacuole (PV) formation by *Plasmodium falciparum* is critical for the development and pathogenesis of malaria. Expansion of the PV membrane (PVM) during growth is orchestrated by the parasite. This is particularly important in mature RBCs, which lack internal organelles and no longer actively synthesize membranes. Pfs16, a 16 kDa integral PVM protein expressed by gametocytes, was chosen as a model for studying the trafficking of material from the parasite across the PV space to the PVM. The locations of Pfs16-green fluorescent protein (GFP) reporter proteins containing distinct regions of Pfs16 were tracked from RBC invasion to emergence. Inclusion of the 53 C-terminal aa of Pfs16 to a GFP reporter construct already containing the N-terminal secretory signal sequence was sufficient for targeting to and retention on the PVM. An aa motif identified in this region was also found in 7 other known PVM proteins. Removal of the 11 C-terminal aa did not affect PVM targeting, but membrane retention was decreased. Additionally, during emergence from the PVM and RBC, native Pfs16 and the full-length Pfs16-GFP reporter protein were found to concentrate on the ends of the gametocyte. Capping was not observed in constructs lacking the aa between the N-terminal secretory signal sequence and the transmembrane domain suggesting that this region, which is not required for PVM targeting, is involved in capping. This is the first report to define the aa domains required for targeting to the *P. falciparum* PVM.

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THE ROLE OF PLASMODIAL CELL CYCLE REGULATOR, PFMRK, IN CELL CYCLE CONTROL AND DNA REPLICATION OF *PLASMODIUM FALCIPARUM*

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Malaria causes approximately one million deaths annually with *Plasmodium falciparum* causing the highest morbidity and mortality. Malaria pathology results from the rapid growth and cyclic multiplication during erythrocytic schizogony. The growth and multiplication is controlled by an unknown cell cycle regulatory mechanism, believed to be similar to mammalian cells. However, there are many features of parasite schizogony that are unique. It is believed that ring stages of *P. falciparum* development are representative of the G1 phase cycle, while late trophozoite and schizont stages are equivalent to S and M phases. The transitions of the parasite through these phases and whether cell cycle checkpoints exist are unknown. In the age of drug resistance and the dire need for novel antimalarial drugs, it is paramount that the plasmodial cell cycle is understood. Cyclin dependent protein kinases (CDKs) are essential regulators for sequential cell growth and proliferation. Pfmrk, a sequence homologue of human CDK7 is suggested to play an important role in both cell cycle control and DNA replication in the plasmodial cell cycle. Pfmrk localises to the nucleus, interacts with DNA machinery and activity peaks during the ring stage development suggesting a role in either G1 or S phase. Parasites treated with Pfmrk inhibitors demonstrated a delayed onset of the second growth cycle and a growth prolongation to develop into mature schizonts. The number of merozoites formed per schizont was reduced approximately fifty percent following the first complete growth cycle. Parasites treated with the DNA synthesis inhibitor, aphidicolin, resulted in a stage-specific alteration of Pfmrk kinase activity. These studies suggest that Pfmrk may function at a specific phase of the cell cycle, presumably early G1. Further investigations using transgenic parasites that over-express either a functional or non-functional Pfmrk kinase revealed that the latter takes longer time to complete the development cycle. 41 mammalian cell cycle inhibitors that affect different aspects of the cell cycle machinery were assessed in parasites. Subsequently, compounds were shortlisted for in-depth studies to characterize the plasmodial cell cycle and roles of Pfmrk. In addition, stage specificity studies will also be conducted to investigate inhibitory effects on parasite DNA replication and growth. A proposed model of the plasmodial cell cycle will be presented.

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IMPACT OF INTERMITTENT PREVENTIVE TREATMENT IN CHILDREN (IPTC) ON *PLASMODIUM FALCIPARUM* INFECTIONS COMPLEXITY: RESISTANCE MARKERS AND KINETIC OF ANTIBODIES AGAINST *P. FALCIPARUM* IN SENEGAL

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Key interventions currently recommended by WHO for the control of malaria are the use of insecticidal treated nets (ITNs) or indoor residual spraying (IRS) for vector control, and prompt access to diagnosis and treatment of confirmed clinical malaria cases. Additionally, WHO is now recommending Intermittent Preventive Treatment for infants (IPTi) against *Plasmodium falciparum* malaria. Currently, a full therapeutic course of sulphadoxine-pyrimethamine (SP) (SP-IPTi) is administered at defined intervals through routine vaccination schedules, such as the Expanded Program on Immunization (EPI), usually at 10 weeks, 14 weeks, and ~9 months of age, to infants living in areas of high malaria risk. Furthermore, the concept is currently being investigated regarding the feasibility of using SP for IPT in children under five years (IPTc). One of the main obstacles to effective malaria control is the emergence of antimalarial drug resistance. Thus, it is crucial to determine the level of SP resistance and how SP-IPTi/c programmes may affect the spread of drug-resistant parasites to ensure continuing high efficacy of SP for IPTi and IPTc. Resistance of *P. falciparum* to SP is due to mutations in the Pfdhfr and Pfdhps genes and most likely mutations in the recently discovered Pfmrp gene may also play a role. Monitoring these mutations will indicate whether IPT-interventions selects for SP resistance. The acquisition of immunity to clinical malaria is usually acquired the first five years of life depending on the intensity of malaria transmission. However, IPT-interventions may hinder such acquisition and postpone when the children are protected and thus cause morbidity in older age groups that previously were protected. Thus, as with any drug-based intervention strategy, it is important to understand how implementation may affect the spread of drug-resistant parasites and host immunity. This study will investigate the possible selection of SP resistance at molecular level after administration of SP for IPTi and IPTc at different times throughout the IPT intervention by comparing infections in infants/children under SP-IPT with those not

receiving SP-IPT. Furthermore, the study will explore whether SP hinder the acquisition of immunity in children under intense infection pressure by comparing the kinetics of specific antibodies against malaria among children under SP-TPI and without SP-IPT.

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PLASMODIUM FALCIPARUM NOVEL RO33 AND 3D7 HAPLOTYPE IS MORE FREQUENT AND DISPERSE IN THE PERUVIAN AMAZON

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Merozoite surface protein1&2 (MSP1/2) gene can express different allelic forms generating polymorphic antigens that confer the parasite the ability to evade the immune response. Hence, it is important to know the genetic diversity displayed by the parasite population to provide new insights for the development of effective malaria control measures. The studies of genetic diversity carried out in areas nearby Iquitos have reported a high frequency of K1 and MAD20 alleles for *Plasmodium falciparum* MSP1. The RO33 allele was previously found in low frequency and only in Zungarococha community, nearby Iquitos city. In this study, besides samples obtained from San Juan community (around Iquitos city), it was also included *P. falciparum* samples from 5 remote communities (Atalaya, Mazan, Requena, Caballococha and Yurimaguas) accessible only by river and away apart up to 396km from Iquitos. 85 *P. falciparum* positive patients, by Microscopy and PCR, were enrolled between 2007 and 2010. The parasite genetic diversity was evaluated by nested PCR using MSP-1/2 allele specific primer. 41/85(48.2%) samples showed the particular RO33 allele in 4 of these communities. From them, 40/41(97.6%) patients showed more symptoms like fever, vomits, weakness, dizziness, arthralgy and muscle pain; suggesting a possible association between the presence of RO33 and the development of these symptoms, compared with the group without this allele. Furthermore, it was found that 18/41(43.9%) patients who had RO33 (MSP1) also had the 3D7 allele (MSP2). This study reports that in the Peruvian Amazon RO33 and 3D7 haplotype is more frequent and disperse, than previously thought, in areas around Iquitos city and far away. Both alleles might contribute to the parasite virulence that finally produced the symptoms observed in these patients. In previous studies in Africa and India both alleles have been associated with the commonest severe complications (cerebral malaria and anaemia). Additional studies would be necessary to address the issue of severe malaria in the Amazon region.

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DEVELOPMENT AND EVALUATION OF A PROTOTYPE NON-WOVEN FABRIC FILTER FOR PURIFICATION OF MALARIA-INFECTED BLOOD

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Many malaria related studies depend on infected red blood cells (iRBCs) as fundamental material, however, infected blood samples from human or animal models include leukocytes, and especially in cases involving *Plasmodium vivax*. These host WBCs are a source of contamination in biology, immunology, and molecular biology malaria studies, requiring their removal. Non-woven fabric (NWF) has the ability to adsorb leukocytes and is already used as filtration material to deplete WBCs for blood transfusion and surgery. Here, we report the development and evaluation of a prototype NWF filter designed for purifying iRBCs from malaria-infected blood. In this study, a total of 15 blood samples of *P. vivax* patients were processed separately by NWF filter and CF11 column methods. WBCs and RBCs were counted, parasite density, morphology and developing stage were checked by microscopy, and compared before and after treatment. The viability of filtrated *P. vivax* parasites was examined by *in vitro* short-term cultivation. The WBC removal rate of the NWF filter method was 99.03%, significantly higher than the CF11 methods (95.48%, $P < 0.01$). The RBC recovery rate of the NWF filter method was 95.48%, also significantly higher than the CF11 method (87.05%, $P < 0.01$). Fourteen *in vitro* short-term culture results showed that after filter treatment, *P. vivax* parasite could develop as normal as CF11 method, and no obvious density, developing stage difference were fund between two methods. In conclusion**,** this new designed NWF filter filtration can remove most leukocytes from malaria-infected blood, and the recovery rate of RBCs is higher than with CF11 column method. Filtrated *P. vivax* parasites were morphologically normal, viable, and suitable for short-term *in vitro* culture. The NWF filter filtration is simple, fast and robust, and is ideal for purification of malaria-infected blood.

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COMPARATIVE ANALYSES OF RECOMBINATION ACTIVITY BETWEEN PFRAD51 AND TGRAD51

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A DNA double strand break (DSB) can be repaired by homologous recombination (HR) or non-homologous end joining (NHEJ) mechanisms. Interestingly, prokaryotes predominantly rely on HR with little or no NHEJ. A similar trend is observed in lower eukaryotes that prefer HR over NHEJ. As we move higher in evolution to mammals, NHEJ is the major repair mechanism and HR is a minor pathway. Interestingly, an apicomplexan parasite, *Toxoplasma gondii* despite being a lower eukaryote leans towards NHEJ. Whereas its sister apicomplexan parasite *Plasmodium falciparum* solely rely on HR mechanism. In order to gain mechanistic insights for such opposite repair choices between two closely related lower eukaryotes, we have begun to characterize HR repair pathways in *P. falciparum* and *T. gondii*. To address whether HR is less efficient in *T. gondii* we have performed genetic analysis in surrogate yeast system. To this end we choose to characterize *TgRAD51* gene. Genetic studies in yeast model system revealed that *TgRAD51* protein is less efficient in handling numerous double strand breaks when compared to *PfRAD51* and *ScRAD51*. In case of a single DSB, the repair efficiencies of these genes were comparable. In order to correlate such inefficient DSB repair activity of TgRad51 to its catalytic activity, *TgRAD51* gene was cloned, expressed in bacteria and purified to near homogeneity. The kinetics of ATPase activity of TgRad51 protein was investigated in comparison to PfRad51 and ScRad51 proteins.

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POLYMORPHISMS OF *PLASMODIUM FALCIPARUM* INFECTION IN AN ASYMPTOMATIC COHORT LIVING IN THE FOREST-SAVANNA ZONE OF GHANA: AGE AND SEASONAL ANALYSES

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Trends in the transmission of malaria at the Forest and Savannah Zones of Ghana are depicted in Kintampo, making it feasible to obtain from one place malaria indices which are reflective of what pertains in both zones of the country. This was part reason for demarcating the Kintampo districts for antimalarials and malaria vaccine trials. Subsequently surveys were conducted to ensure that malaria indices were characterized prior to trials. The multiplicity of infection (MOI) within asymptomatic children and adults; the distribution of the MOI in different age categories during

the different seasons of the year and the molecular dynamics of MOI is reported. Study area was divided into sixteen clusters and asymptomatic residents were identified. Resident participants, aged between 3 weeks and 78 years, were followed-up on a two-month rotation for a year. On each follow-up visit, 2 blood samples were collected onto blood slides and filter papers for microscopy and genoytping respectively. Filter papers of 600 (100 from each rotation) samples which were microscopically positive were randomly selected for genotyping. A mean MOI of 7 was most frequent between January and April, while the least and most frequent MOI of 1 was observed in November/December. Children had MOI averaging between 7 and 8 while adults had MOI averaging between 2 and 3. Children below five years had highest mean MOI of 7 in the March/April survey and lowest mean MOI of 2 in the May/June survey. Participants older than 5 years had highest mean MOI of 3 in the March/April survey and lowest mean MOI of 1 in May/June. Also, during March/April, IC/3D7 out-numbered FC27 strains by a ratio of 2:1. In other seasons however, this ratio increased to 4:1. Both antigenic variants peaked during March/April, and were at their lowest numbers during September/October. MOI as observed in this study demonstrates that malaria transmission is high in Kintampo. The relatively higher number of infections in younger participants than older participants reflects a possible association between asymptomatic status and carriage of high non-virulent parasite strains in children. IC/3D7 strains occurred most frequently in this asymptomatic cohort at all times; suggesting that IC/3D7 could be a non-virulent strain in this cohort. Further analyses also showed that the potential of this asymptomatic cohort to harbour and transmit *P. falciparum* resistant parasites was highest between January and April.

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DETECTION OF GENOTYPICALLY IDENTICAL PARASITES IN THIES, SENEGAL AFTER APPLICATION OF INTERVENTION STRATEGIES

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Recent efforts to combat malaria have shifted emphasis from control to regional elimination and global eradication of malaria. Such a campaign requires tools to monitor genetic changes in the parasite that compromise the effectiveness intervention efforts. Using a previously described molecular barcode (Daniels, 2008) for *Plasmodium falciparum* that allows unique identification of parasite strains, we applied the technology to filter paper samples derived from patients seen at clinic for malaria treatment in Thies, Senegal from 2006-2010. Starting in 2008 the National Malaria Control Program in Senegal applied a number of intervention strategies at the Thies site including indoor residual spraying and bednet use (WHO, 2010). When we assessed parasite samples from this site before and after these intervention strategies were applied, we observed an unexpectedly high number (25%) of parasites that appeared genotypically identical to one another in 2008, suggesting a severe bottleneck that reduced the effective population size. When we tracked parasites across transmission seasons, we observed evidence of genotypically identical parasites in the next year and beyond. Genotypically identical parasites were confirmed to be from independent human infections with no detectable familial or residential relationship in multiple years. In addition, when applied to our high-density SNP genotyping array, these samples were indistinguishable from one another. We found no genotypically identical parasites among similar samples obtained in Malawi, where transmission intensity is greater and control strategies are different. Further, when applied to a global set of more than 400 parasites, we found no matching molecular barcodes. These data suggest that the molecular barcode can monitor parasites in the natural setting and detect changes in population structure either over time or as a consequence of intervention strategies. This method may be a useful proxy for transmission as it can directly assess which parasites are successfully transmitted during natural infection.

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PHASE I/IIA CLINICAL EVALUATION OF THE EFFICACY OF NEW VIRAL VECTORED VACCINES TARGETING THE *PLASMODIUM FALCIPARUM* BLOOD-STAGE ANTIGENS: MSP1 AND AMA1 IN MALARIA NAÏVE INDIVIDUALS

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Viral vectored vaccines encoding blood-stage malaria antigens MSP1 and AMA1 can stimulate potent cellular and humoral immune responses in mice and rhesus macaques and induce protective efficacy in rodent malaria models. We sought to test the safety, immunogenicity and efficacy of this approach in a Phase I/IIa sporozoite challenge trial using the simian adenovirus 63 (AdCh63) and poxvirus MVA vectors (administered in a heterologous prime-boost regimen) encoding MSP1 (n=10), AMA1 (n=9) or both antigens co-administered (n=9). The MSP1 antigen included conserved blocks of sequence and both alleles of the 42kDa C-terminus. The AMA1 antigen included the two divergent alleles of AMA1 (3D7 and FVO) in tandem. The vaccines were safe and immunogenic, inducing high level antibody responses and the strongest T cells responses yet reported by subunit vaccination (as measured by ex-vivo IFN- γ ELIspot assay). Co-administration of AMA1 and MSP1 vaccines was associated with a reduction in the total T cell and antibody responses to each individual antigen when compared to single vaccine administration, but was associated with protective clinical efficacy against 3D7 strain *Plasmodium falciparum* sporozoite challenge (1/9 volunteers demonstrating sterile protection and two others demonstrating delay to parasitaemia diagnosis by thick-film microscopy). One volunteer receiving AdCh63-MVA AMA1 demonstrated a substantial delay in time to patent parasitaemia, whilst volunteers receiving AdCh63-MVA MSP1 demonstrated no clinical efficacy. This AdCh63-MVA viral vectored vaccine platform provided some detectable efficacy, with the first sterile protection of any vaccinee using blood-stage malaria antigens alone. This provides evidence that vaccines inducing cell-mediated responses in conjunction with antibody responses to the blood-stage antigens MSP1 and AMA1 are safe as well as partially effective. This vaccine delivery technology provides a versatile and reliable approach for the development of new vaccines against other infectious diseases where both strong cellular and humoral immunity are likely required for protective efficacy.

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SAFETY AND IMMUNOGENICITY OF HETEROLOGOUS PRIME-BOOST WITH THE CANDIDATE MALARIA VACCINES ADCH63 ME-TRAP AND MVA ME-TRAP IN HEALTHY ADULTS IN A MALARIA ENDEMIC AREA

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Malaria is estimated to cause nearly a million deaths and upto to five hundred million cases annually.It is accepted that current control tools may not be sufficient to address the malaria burden in many contexts; therefore there is a need for additional interventions and an efficacious malaria vaccine would go a long way in this fight. We report a phase one b trial of viral vectored candidate malaria vaccines AdCh63 ME -TRAP and MVA ME- TRAP.Thirty consenting healthy male volunteers were recruited and randomized either low or high dose AdCh63 ME-TRAP prime and MVA ME-TRAP boost either intradermally or intramuscularly.The local solicited symptoms reported were swelling, itch, warmth, pain, redness, scaling and blistering while the general symptoms were fever, headache, arthralgia, myalgia, nausea/vomiting and malaise. All the symptoms reported post vaccination were mild to moderate in nature and have all since resolved. There is no significant difference between the intramuscular and intradermal routes of vaccine administration at all timepoints. The highest peak response response was at day sixty three with an arithmetic mean for the the high dose groups of one thousand seven hundred and ninety two; with a ninety five percent confidence interval of one thousand two hundred and twenty nine to two thousand three hundred and fifty five and a p value of zero point nine seven.We confirm that this approach is safe and immunogenic by measurement of interferon γ responses. There is no significant difference between the groups.

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ANTIBODY-MEDIATED RESPIRATORY BURST AS AN IMMUNE CORRELATE OF PROTECTION IN MALARIA VACCINE DEVELOPMENT

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Malaria is a leading cause of morbidity and mortality in the tropics. The development of an effective vaccine is vital for the control of the disease and remains a challenge. Several promising novel antigen formulations and platforms are in clinical evaluation. However, there is still lack of robust assays that can be used as immune correlates of protection in vaccine trials and the few that are available have not demonstrated consistency in predicting the hypothesized outcomes when tested in the field. Based on the notion that the process of respiratory burst by innate cells that lead to their production of reactive oxygen species (ROS) have been associated with immunity to malaria as previously observed in population based studies, we postulate an ex vivo assay to quantify antibody-mediated respiratory burst activity with peripheral effector cells. Using merozoites isolated from enriched malaria cultures, we are able to test different panels of antibodies in terms of their ability to opsonize and form potent merozoite-coated complexes that trigger the production of ROS in the presence of either neutrophils or monocytes. Using an isoluminol-amplified chemiluminescence technique to quantify ROS, we can evaluate the functional capacities of antibodies based on their ROS profiles. This assay could be an important platform to interrogate different malaria vaccine candidates in terms of their effectiveness at raising relevant antibodies that mediate parasite clearance by triggering the peripheral effector cells to generate reactive oxygen radicals that may be a characteristic of vaccine-induced protection.

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IMMUNOGENICITY OF NANOPARTICLE-COATED MSP-1 C-TERMINUS MALARIA DNA VACCINE USING DIFFERENT ROUTES OF ADMINISTRATION - MURINE MODEL

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In malaria DNA vaccination, alternative delivery systems having higher transfection efficiency and eventual superior antibody production needs to be further explored. On the other hand, identification of optimal route of administration to enhance humoral and cellular immune responses is believed to be an important step in the development of vaccines against malaria infection. In this study, the effect of nanoparticle coating on *Plasmodium yoelii* MSP1-c-terminus plasmid on induction of immune response in mice was examined. Groups of C57BL/6 mice were immunized either with nanoparticle-coated plasmid or naked by using three different routes of administration (i.v. i.p. and s.c.). To evaluate the protection level, mice were challenged with 10^5 of *P. yoelii*-infected red blood cells, two weeks after the last immunization. Measurement of IgG and its subclass antibody titer by ELISA showed higher titer in coated group than the naked group. Flow cytometric analysis of splenic cells after immunization with coated DNA showed an increased proportion of both CD4+and CD8+ subpopulation of T cells. Cytokines levels in the culture supernatant of merozoite antigen-stimulated splenocytes and sera were observed to be significantly higher in the coated as compared to naked or control group. High levels of Th1 and Th2 types of cytokines were observed in vaccinated mice by i.p. followed by i.v. than s.c. vaccinated mice. As well as INF-γ ELISPOT producing cell number of splenocytes, indicated some stimulatory effect of this novel nanoparticle on coating MSP1 DNA vaccine and might have enhanced the protective immunity against blood stage malaria. In all the three different routes of administration, nanoparticle coating substantially enhanced IgG response, CD4+and CD8+T cell populations, cytokine induction and protection. Better protection by route of administration was observed to be in the following order i.p.>i.v.>s.c.

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AN ALL-SYNTHETIC NANOSPHERE VACCINE TARGETING *PLASMODIUM FALCIPARUM* ENOLASE INDUCES PROTECTIVE EFFECT AGAINST *P. BERGHEI* MALARIA IN MICE

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Enolase catalyzes at the ninth step of the eleven enzymes in the glycolytic pathway. Our field serological studies have suggested that antigens toward *Plasmodium falciparum* enolase were strongly presented by the sera taken from endemic inhabitants who have present and/or recent past infection. To use our findings for vaccine development, we have designed an all-synthetic vaccination material to realize the immunity condition in endemic area, in which residents are sequentially infected and thus sustain immunity against parasite infection. Previously we have reported nano-encapsulation of a synthetic antigenic peptide AD22 based on the enolase and the immunological properties of nanospheres. In this paper, we wish to present the protective effect against *Plasmodium berghei* malaria induced byimmunization with a nanosphere material. Nanosphere Preparation. The nanospheres were formulated using an oil/water emulsion technique with a bioabsorbable polymer, poly(lactic acid-*co*-glycolic acid). The antigen content was adjusted to 20 mg/mg of the material. Mice were immunized by subcutaneous injection of 2.5 mg nanoparticle (50 mg antigen) at 21-day intervals three (Day-0, -21, and -42). After three immunizations, the antibody titers against *Pf* AD22 were monitored. The antibody response of the mice was 20-fold increase at Day-49 if the IgG titer was compared with non-encapsulated control.

Mice having mean anti-*Pf* AD22 antibody titers of 1:5,200 were then challenged with the lethal strain of *P. berghei* (strain ANKA; 10^6 parasites per mouse), and parasitemia was monitored weekly. Since *Pf* and *Pb* AD22 sequences show 90% sequence homology and since antibodies to *Pf* AD22 cross-react with *Pb* enolase in the erythrocytic stages of the parasite, we examined the effect of immunization of mice with *Pf* AD22. (4) Data Analysis. The average parasitemia and survival pattern for each group of mice are monitored over a period of 24 days after the challenge infection. Among the mice immunized with the nanosphere, there was slower initial increase in parasitemia by Day-5 post-challenge, and the peak parasitemia was about 60% on Day-23 post-challenge. The survival profile have shown that the immunized mice had a significantly longer survival period compared with PBS-immunized negative control which shows the peak parasitemia on Day-14 post-challenge.

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ANALYSIS OF CELL-MEDIATED IMMUNE RESPONSES AND DIFFERENCES IN PROTECTIVE EFFICACY OF AN ADENOVIRUS-VECTORED *PLASMODIUM FALCIPARUM* MALARIA VACCINE WITH AND WITHOUT DNA PRIMING

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We tested a DNA prime / serotype 5 adenovirus boost *Plasmodium falciparum* malaria vaccine in 15 malaria-naïve adults. Both the DNA and Ad5 vaccines encoded the circumsporozoite protein (CSP) and apical membrane antigen-1 (AMA1). This heterologous regimen sterilely protected 4 of 15 volunteers against malaria sporozoite challenge, while two other trials lacking DNA priming were not protective, one a single dose of Ad5 CSP and AMA1 (AdCA), the second 2 doses of AdCSP (AdC). Because IFNγ secreting CD8+ T cells have been implicated in protection against pre-erythrocytic stage malaria, we assessed IFNg secretion from peripheral blood mononuclear cells stimulated with CSP and/or AMA1 peptide pools, particularly since antibody responses were poor. In the DNA prime-Ad boost trial (D-AdCA), IFNγ ELISpot assays demonstrated that 2 of the 4 protected volunteers developed robust responses to specific single CSP peptide pools, and 3 of the 4 to specific single AMA1 pools. These protected volunteers made almost no significant response against other peptide pools. The fourth protected volunteer had poor ELISpot responses to both antigens. ELISpot responses of non-protected volunteers were more widely distributed among all CSP and AMA1 peptide pools but were comparatively low in the DNA/Ad trial. In contrast, volunteers in the AdCA alone trial developed robust responses to multiple CSP and AMA1 peptide pools, with magnitude of summed responses exceeding those induced in the protected volunteers in the D-AdCA trial, with similar results in the AdC trial. This indicated a qualitative improvement in responses associated with DNA priming, even though quantitative responses were lower. While ICS assays are ongoing, preliminary ELISpot depletion assays in 2 of the 4 protected volunteers identified dependence on HLA-restricted CD8+ T-cell-mediated response, suggesting that the DNA prime / Ad boost malaria vaccine may be the first gene-based subunit vaccine to induce protection against any pathogen in humans via HLA-restricted T-cell-mediated immunity.

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PRIME-BOOST COMBINATIONS OF DNA SUBUNIT VACCINES AND RADIATION-ATTENUATED SPOROZOITES FOR IMPROVING PROTECTION AND IDENTIFYING NOVEL PROTECTIVE PRE-ERYTHROCYTIC STAGE ANTIGENS

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We hypothesized that a prime-boost immunization regimen that combines a subunit malaria vaccine consisting of a single *Plasmodium yoelii* sporozoite test antigen with a suboptimal (partially protective) dose of radiation-attenuated *P. yoelii* sporozoites (*IrrPy*Spz) (presumably inducing responses to scores of unidentified sporozoite proteins) will significantly enhance protection compared with either the subunit vaccine or *IrrPy*Spz administered alone, whenever the test antigen is one of the antigens boosted by *IrrPy*Spz. This approach should provide a sensitive screening method for revealing the protective potential of novel sporozoite antigens. Demonstrating enhanced protection would indicate that the novel test antigen likely contributes to the protection induced by *IrrPy*Spz and that the *P. falciparum* ortholog could be a suitable candidate for clinical development. Our initial suboptimal *IrrPy*Spz experiments utilized 2 doses of 2K *IrrPy*Spz, which consistently improved protection from a moderate level, 58% (7/12), to 83% (10/12) if mice were first primed with a non-protective dose of *Py*CSP plasmid DNA. We then moved from the *IrrPy*Spz two-dose regimen to a one-dose regimen of 20K or 30K *IrrPy*Spz, and again, protection improved from a moderate level of 42% (5/12) induced by the *IrrPy*Spz alone to 75% (9/12) and 100% (12/12) in two studies when mice were first primed with a non-protective dose of *Py*CSP plasmid DNA. The single dose *IrrPy*Spz regimen has subsequently been used to screen and rank the protective potential of 5 new *Py* orthologs of *Pf* antigens in five groups of mice. The protection obtained after the booster dose of 20K *IrrPy*Spz was 18%, 45%, 54%, 80%, and 90% respectively when 5 new antigen test groups were each primed with a non-protective plasmid DNA regimen, compared to 18% (2/11), when priming was done with empty plasmid. The reproducibility of these results, the mechanism of protection (antibody and T-cell response measurements), and the magnitude and longevity of the protective immune response are currently being investigated and will be presented.

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TARGETING SIALIC ACID-DEPENDENT AND -INDEPENDENT PATHWAYS OF INVASION IN *PLASMODIUM FALCIPARUM*

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The pathology of malaria is a consequence of the parasitemia which develops through the cyclical asexual replication of parasites in a patient's red blood cells (RBC). Multiple parasite ligand-erythrocyte receptor interactions must occur for successful *Plasmodium* invasion of the human red cell. Two major malaria ligand families have been implicated in these variable receptor-ligand interactions used by *Plasmodium falciparum* to invade human erythrocytes. The micronemal proteins form the *ebl* family (for *e*rythrocyte *b*inding *l*igands) and the rhoptry proteins form the Reticulocyte binding Homolog (Pf*RH)* family. Ligands from the ebl family largely govern the sialic acid (SA) dependent pathways of invasion and the RH family ligands (except for RH1) mediate SA independent invasion. In an attempt to dissect out the invasion inhibitory effects of antibodies against ligands from both pathways, we have used EBA-175 and RH5 as model members of each pathway. Mice were immunized with region II of EBA175 produced in *P. pastoris*, full length RH5 produced by the wheat germ cell-free system and combinations of the two antigens, to

look for synergistic effects. Sera obtained from these immunizations were tested for native antigen recognition and for efficacy in growth inhibition assays. Results obtained show promise for the potential use of such hybrid vaccines to induce antibodies that can block multiple red cell receptor-parasite ligand interactions and thus inhibit parasite invasion.

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IDENTIFICATION OF A NOVEL *PLASMODIUM FALCIPARUM* MEROZOITE MICRONEMAL PROTEIN AS A BLOOD-STAGE VACCINE CANDIDATE

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One of the solutions for reducing the global mortality and morbidity due to malaria is multivalent vaccines comprising antigens of several lifecycle stages of the malarial parasite. Hence, there is a need for supplementing the current set of malaria vaccine candidate antigens. Here, we aimed to characterize GPI-anchored micronemal antigen (GAMA) encoded by PF08_0008 gene in *Plasmodium falciparum*. Antibodies were raised against recombinant GAMA synthesized using wheat germ cell-free system. In western blotting, anti-GAMA antibodies reconfirmed processing and shedding of GAMA. Immunofluorescence assays showed that GAMA is initially localized in the apical region of merozoites of mature schizonts and later relocated onto the surface of free merozoites. Immunoelectron microscopy demonstrated for the first time that GAMA is a microneme protein of the merozoite. Erythrocyte binding assays revealed that GAMA possesses an erythrocyte binding epitope in the C-terminal region. GAMA binds erythrocytes in a neuraminidase resistant and chymotrypsin sensitive manner suggesting that GAMA may represent a sialic acid-independent ligand. In growth inhibition assays, anti-GAMA antibodies inhibited *P. falciparum* invasion in a dose dependent manner. Additive blocking of invasion exhibited by mixing of anti-GAMA and anti-EBA175 antibodies suggests that targeting of both sialic acid-independent and sialic acid-dependent ligands is more effective than targeting either ligand alone. Human sera collected from endemic areas of Mali and Thailand recognized GAMA suggesting that GAMA is immunogenic to humans. Since GAMA in *P. falciparum* is refractory to gene knockout attempts, it is essential to parasite invasion. Overall, our study indicates that GAMA is a novel blood-stage vaccine candidate antigen.

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IN VIVO WHOLE BODY IMAGING OF MICE FOR ASSESSMENT OF THE EFFICIENCY OF LIVER STAGE INFECTION AFTER PARENTERAL ADMINISTRATION OF *PLASMODIUM BERGHEI* SPOROZOITES

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Pre-clinical protection studies with whole organism malaria vaccine candidates in mice are generally performed by intravenous immunization of sporozoites. Although highly efficient for infection and induction of protection, intravenous administration of parasites is not the preferred route for human vaccination. Studies in mice with intradermal and subcutaneous immunization regimens showed a strong decrease in protective efficacy against malaria compared to intravenous immunization. Using bioluminescent *Plasmodium berghei* sporozoites, we more recently found that the decrease in protective efficacy, associated with a decreased parasite liver infection. The objective of this study was to explore alternative routes of parenteral sporozoite administration for high efficiency of liver infection. As determined by *in vivo* whole body imaging, we found that the route of administration, the location of injection and the volume in which sporozoites are administered, have significant effects on the subsequent degree of parasite liver load development. We will present a protocol for administration of sporozoites other than intravenously that leads to liver stage infection, sufficient for the induction of protection.

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IMMUNO SCREENING OF *PLASMODIUM YOELII* PRE-ERYTHROCYTIC ANTIGENS FOR MALARIA VACCINE DEVELOPMENT

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Malaria is a disease caused by protozoan parasites from the genus *Plasmodium* transmitted by the bites of *Anopheles* mosquitoes. Infectious sporozoites travel to the liver via the blood stream, invade hepatocytes and develop innocuously over several days. Released back into the blood as merozoites, they invade erythrocytes resulting in anemia and clinical disease. Although there is little evidence that immunity to the pre-erythrocytic stages develops naturally following exposure in endemic areas, experimental immunization of animals or humans with radiation-attenuated sporozoites (RAS) renders sterile protection for variable periods. This protection is mediated by cellular responses targeting as yet unknown pre-erythrocytic stage antigens, the identification of which could lead to the development of a highly effective malaria vaccine. Thus far only a handful of pre-erythrocytic antigens, representing less than 0.3% of the proteome, have been tested, and only one, CSP, has been shown to contribute to the protection afforded by RAS. Here, we report a study aiming to discover additional pre-erythrocytic antigens capable of i) recalling responses in splenocytes from RAS-protected mice, and ii) protecting mice against sporozoite challenge. A panel of 150 sporozoite and liver stage antigens was selected based on high expression profiles in transcriptome and proteome databases. *P. yoelii* genes were cloned into the DNA vaccine vector VR1020-DV and interferon-gamma (IFNγ) ELISpot screening assays were performed using splenocytes stimulated with A20 antigen-specific presenting cells. Recombinant proteins were also screened for reactivity to sera from RAS-immunized mice. Protection studies are now being carried out to test the top 10% antigens as ranked by ELISpot screening, immunizing with DNA delivered intramuscularly by electroporation using the Ichor TriGrid™ device. Upon sporozoite challenge, protection is assessed by both RT-PCR to measure liver-stage parasite burden and by blood stage parasitaemia. Data on the identification of antigens, immunoscreening and protection will be presented.

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PROTECTION OF PYCSP VACCINE IS ENHANCED BY INCLUSION OF TWO NEW *PLASMODIUM YOELII* VACCINE ANTIGENS

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Despite years of effort, a licensed malaria vaccine is not yet available. The most advanced malaria vaccine candidate, RTS,S, is currently being evaluated in a phase 3 trial at eleven sites in seven African countries. RTS,S is a recombinant protein vaccine based on the *Plasmodium falciparum* circumsporozoite protein (PfCSP). It has protected malaria-naïve adults against an experimental *P. falciparum* challenge and reduced malaria-associated episodes in children living in malaria endemic areas. The level and duration of immunity induced by RTS,S, however, is relatively modest. Therefore, even if RTS,S is approved for use in infants in malaria endemic regions, a more potent malaria vaccine is needed. One way to potentially enhance the efficacy of RTS,S, or any other subunit malaria vaccine, is to incorporate additional malaria antigens into the vaccine. If RTS,S is part of an established vaccine regimen, any subsequent malaria vaccine may need to include RTS,S or an equivalent PfCSP vaccine component. We have previously shown that a vaccine with two new *P. yoelii* antigens, PyUIS3 (PY3011) and PY3424 (a *P. yoelii* falstatin ortholog), can protect mice against a *P. yoelii* challenge. We now show that bivalent or trivalent vaccines that combine PyCSP, PyUIS3 and PY3424 can protect a higher percentage of mice against a *P. yoelii* challenge than any of the single antigen vaccines alone.

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PRECLINICAL DEVELOPMENT OF A COMBINED VACCINE AGAINST BLOODSTAGE *PLASMODIUM FALCIPARUM* MALARIA

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Invasion of erythrocytes and the cycles of growth of blood stage parasites are central features of the virulence and pathogenicity of *Plasmodium falciparum*. Vaccination against the blood stage of parasite development is a key strategy in alleviating the global burden of malaria. This presents a major challenge to researchers not least because *P. falciparum* has evolved a complex series of alternative erythrocyte invasion pathways mediated by multiple ligands expressed at the point of merozoite-erythrocyte contact. Many of these invasion ligands belong to the erythrocyte-binding like family (EBLs) or the reticulocyte-binding like homologues (RBLs). Evidence for these proteins playing important roles in different erythrocyte invasion pathways has mounted and the creation of genetic mutants lacking one or more of these molecules has proven extremely useful in their characterisation. However, this has also served to illustrate the redundancy of individual proteins in the overall ability of *P. falciparum* to complete its cycle of invasion and growth at least *in vitro*. There now exists a body of evidence suggesting that the EBLs and the RBLs are functionally equivalent in that members of both families bind directly to erythrocytes and are able to mediate invasion. We are investigating whether antibody targeting multiple EBL/RBL molecules can overcome the plasticity of Pf invasion, inhibiting a wider spectrum of parasite invasion pathways.

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NOVEL WAY OF DISSEMINATING ENTOMOPATHOGENIC FUNGI: INFECTION POTENTIAL IN THE WILD *ANOPHELES ARABIENSIS* MOSQUITOES USING CATTLE SPRAYED WITH *METHARIZIUM ANISOPLIAE* IP46

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Improved fungal formulations and effective delivery techniques are prerequisites for successful implementation of entomopathogenic fungi as malaria vector bio-control strategy. The entomopathogenic fungus, *Metarhizium anisopliae* IP46, is known to cause reductions in survival and significant mortality in wild malaria vectors. In the present study, we demonstrated that both calves and their huts sprayed with *Metarhizium anisopliae* IP46 conidia (5×10^{10}conidia/m^2) can result in high fungal infection and significant mortality in the wild *Anopheles arabiensis*. This field experiment, in a rural village of Kilombero valley, Tanzania, showed that more than 71% of all exposed mosquitoes died within 12 days post-exposure. A range of 70-90% fungal infections was recorded in the mosquito cadavers 5-6 days post-incubation. Regardless of mosquito blood-feeding status, significant reductions in daily survival was observed in all treatments combinations. These results strongly suggest that the use calf/cattle either as the bait or contamination source for fungal pathogens can result in high infection rates in wild malaria vectors *An.arabiensis* and conceivable malaria transmission interruption. But also, these findings highlight the possible use of this technique for auto dissemination of potent larvicides and pupacides in the field.

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THE SEGREGATION AND ASSORTATIVE MATING OF BREEDING SWARMS OF *ANOPHELES GAMBIAE* COMPLEX IN MALARIA CONTROL PERSPECTIVE

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Malaria causes a considerable burden for human heath in sub-Saharan Africa. Resistances to drugs and insecticides have challenged the approaches to control malaria. The GMM and SIT constitute alternative strategies for malaria vector control. Both approaches require a fine understanding of the biology of reproduction *Anopheles gambiae* sl, including the main malaria vectors in Africa. The objective of this study was to characterize the swarm structure of two sibling species, *An. gambiae* ss and *An. melas* and to explore the ecological and environmental parameters associated with the formation of swarm and their spatial distribution. After the survey at Djègbadji, in the coastal lagoon of Southern Benin, January to December 2010, swarms and breeding sites were searched and sampled and identified by RFLP-PCR. Swarm sites, human dwellings and breeding sites were integrated in the same satellite image using GIS and were analyzed. During the dry season period, 34 swarms of *An. gambiae* sl were sampled from 17 swarming sites yielding a total of 6,864 males and 12 females. These 17 swarming sites, 8 were composed exclusively of *An. gambiae* referring to M form and 9 for *An. melas*, evidence of breeding swarm segregation. Nevertheless the two species exhibited differences through the swarm size, the swarming height and mating events observed. The couples sampled from swarms were assortative mating. The swarming site localization was close to human dwellings for the M molecular form of *An. gambiae* and on salt production site for *An. melas*. *An. gambiae* swarms were closer

to human dwellings than their breeding sites. During the rainy season period, *An. melas* breeding sites disappeared because of the flooding and additional breeding sites of *An. gambiae* are created within the village. At the peak of rainy season, swarm of *An. melas* was absent but four additional swarming sites of *An. gambiae* M form have been recorded with increase of the swarm size. These findings offer evidence that the ecological speciation is associated with spatial swarm segregation and assortative mating, providing strong support for the hypothesis that mate recognition is currently maintaining adaptive differentiation and promoting ecological speciation. Further studies on the swarming and mating system of *An. gambiae* molecular forms in the prospect to produce a predictive model of swarm distribution are needed to better perform in the future any strategies based on the use of GMM and SIT.

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SPATIAL CLUSTERS OF MALARIA INCIDENCE IN YUNNAN, CHINA

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Malaria is a significant public health issue in Yunnan province, China. It is vital to identify high risk areas of malaria and to allocate public health resources properly and effectively. This research aims to detect spatial clusters of malaria incidence at a township level to assist the malaria elimination program (2010-2020) in China. Data on the incidence rates of *Plasmodium falciparum* (P.f) and *P. vivax* (P.v) malaria were calculated in 1602 townships in 2010. Discrete Poisson model and purely spatial analyses were performed to identify high risk clusters using SaTScan software. Disease mapping was conducted using ArcGIS software. The most likely high risk clusters of P.f malaria cases (115 townships) were identified in western Yunnan along China-Myanmar border areas (Relative Risk (RR):79.34) and the secondary clusters were in south-eastern Yunnan along China-Vietnam border areas (RR:16.03). The most likely high risk clusters of P.v (175 townships) cases were identified in western Yunnan along China-Myanmar border areas (RR:28.73) and the secondary clusters (RR:10.68, 13.90 and 6.35) were in north-eastern and south-eastern (China-Vietnam) Yunnan. The identification of high risk locations in Yunnan may provide useful information for the malaria elimination program in China and assist further research to explore key determinants of high risk clusters for malaria transmission in this endemic region.

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THE IMPACT OF HOST HEMATOLOGICAL VARIATION ON THE FITNESS OF THE MALARIA VECTOR *ANOPHELES GAMBIAE* S.S AND ITS CAPACITY TO TRANSMIT *PLASMODIUM FALCIPARUM*

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Anaemia is a common health problem affecting women and children in the developing world. This condition is characterized by a reduction in red blood density, primarily resulting from malnutrition and/or infectious diseases such as malaria. As red blood cells are the most important source of protein for mosquitoes, any reduction could impede the ability of mosquito vectors to transmit malaria by: (i) influencing mosquito longevity (ii) reducing mosquito fecundity and/or (iii) altering the probability of mosquito infection. The aim of this study was to determine how variation in the red cell density of human blood characteristic of that associated with anaemia influences the fitness of *Anopheles gambiae* s.s. vectors and the *Plasmodium falciparum* parasites they carry. Human blood containing gametocytes of the malaria parasite *P. falciparum* of either normal Packed Cell Volume (50%) or that typical of a severely anaemic patient (15% PCV) was fed to groups of *An. gambiae* s.s. females using a membrane feeder. In all experiments, mosquitoes feeding on low PCV blood obtained a significantly lower mass of blood protein than those feeding on blood with normal PCV (X^2_1= 13.96, P< 0.001). However despite this reduction in protein intake, mosquitoes that fed on low PCV blood produced a greater number of eggs than those fed on blood with normal PCV (X^2_1= 35.11, P< 0.001). Risk of death after feeding on blood with normal PCV was approximately 20% lower than after feeding on blood with low PCV (Odds ratio= 0.82, 95% CI:0.72- 0.93). Standardizing for gametocyte density, the oocyst infection rate of mosquitoes fed on blood of low PCV was significantly higher than in mosquitoes fed blood of normal PCV (X^2_1= 7.64, P< 0.001). These results demonstrate that host haematological variation of the magnitude likely to arise in malaria endemic settings may have a significant impact of the outcome of vector -parasite interactions, and that conditions such as anaemia which reduce red blood density could enhance vectorial capacity by increasing parasite infectivity, and mosquito reproduction.

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THE EFFECTS OF INGESTED HUMAN INSULIN ON NF-κB ACTIVATION AND THE MOSQUITO IMMUNE RESPONSE TO MALARIA INFECTION

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NF-κB transcription factors are a critical component of the mosquito innate immune response to a variety of infectious agents, including malaria parasites. In mammalian cells, the insulin/IGF-1 signaling (IIS) cascade can both positively and negatively impact immunity via the regulation of NF-κB and Toll signaling. We have previously shown that ingested human insulin can activate IIS and enhance *P. falciparum* infection in mosquitoes in the lab. We have also shown that mutations in IIS and Toll signaling genes are in linkage disequilibrium and associated with *P. falciparum* infection in field-collected *A. gambiae*. These data suggest that IIS regulation of Toll/NF-κB signaling can alter natural parasite infection. To test this hypothesis, we examined the effects of IIS activation on NF-κB-dependent signaling in mosquito cells *in vitro* and *in vivo*. Our studies have confirmed that distinct pathways of IIS can influence the regulation of NF-κB-dependent mosquito immune responses. The identification of central regulators of anti-parasite immunity, such as the IIS, is a necessary first step towards generating mosquitoes that are refractory to malaria infection.

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A REPEATED THEME - MALARIA OUTBREAKS IN THE MILITARY: CAN WE FIX IT?

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United States (US) military combat, humanitarian assistance in malaria endemic countries expose forward deployed troops and travelers to the risk of contracting malaria and other infectious diseases that can significantly and negatively impact operations and personal travel. The military from all countries have had their battles with malaria and compliance to antimalarial personal protective measures (PPM) and prophylaxis. During World War II, more casualties occurred from malaria than battle. While forces in the Pacific suffered 40% incapacitation due to malaria, there were five-fold greater malaria related casualty rates in Papua New Guinea and Guadalcanal. Unfortunately the lessons learned from these outbreaks were not amended. In 2003, 80 US Marines came down with a febrile illness in Liberia, Africa, 36 were treated presumptively, 39 uncomplicated malaria, 5 complicated malaria. In 2009, 15 US Marine Reservists were treated for malaria from Benin, Africa, 3 presumptively, 11 uncomplicated malaria, 1 complicated malaria. Both of these recent outbreaks had a breakdown in compliance with their personal protective measures (PPM), and antimalarial chemoprophylaxis. Person-days lost does not only interferes with mission readiness, but the medical costs are 35 to 300+ times more than the cost of PPM for each person in an endemic malaria region. Questionnaires revealed the most common reason for not

taking prophylaxis or PPM, was 'forgot'. There have been great advances in working towards a malaria vaccine, but nothing ready to be used any time soon. I would like to present a couple of preventive campaigns to educate and increase our service-members awareness giving them an active role in their health. 1) Personal Protective Packs (PPPs) - premade, designed for different environmental settings: Tropical PPPs: sanitation wipes, DEET, sugar-free gum, and educational comic strips on protection from vector-borne illnesses. 2) Meals Ready to Eat - include the comic strips related to food-borne illnesses. 3) Using information technology (IT) to automatically detect region specific information and deliver PPPs to areas of interest, especially those forward deployed who may not be near the main unit or medical unit nearby. The views expressed in this article are those of the author and do not necessarily reflect the official policy or position of the Department of the Navy, Department of Defense, nor the U.S. Government."

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SECRETION OF ANTI-MALARIAL PROTEINS BY NOVEL SIGNAL PEPTIDES IN *ASAIA BOGORENSIS*

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Three anti-malarial antibodies have each been converted into a single ORF containing the variable region of the antigen binding domain, creating single-chain fragment antibody genes (scFvs). These scFvs target *Plasmodium falciparum* proteins Pfs25, Pfs45/48 and Pfs230 which are necessary for the life cycle of the parasite within the mosquito midgut. The scFv genes have each been cloned into 13 different expression vectors in frame with unique predicted secretion signal peptides. The peptides were identified from secreted proteins in two bacterial species, *Gluconobactger oxydans* and *Gluconacetobacter diazotrophicus*, which are both closely related to *Asaia bogorensis*, a species known to inhabit the mosquito midgut. Plasmid constructs have been tested for secretion of the protein from *Asaia*. Those strains which were positive for secretion were then fed to mosquitoes and tested for transmission-blocking against an infective *P. falciparum* bloodmeal in the mosquito midgut.

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LONG-LASTING INSECTICIDE TREATED BED NETS IN ZAMBIA: HOW LONG ARE THEY LASTING?

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Long-lasting insecticide treated nets (LLINs) are a mainstay of malaria prevention in Africa. More LLINs are available now than in any time previously due to increases in funding for malaria control. LLINs are expected to last three years before being replaced. Reports of nets lasting much less time are frequent in Zambia and may impact estimates of LLINs needed to achieve universal coverage. This study collected nets distributed in mass distribution campaigns and by local caregivers. One net was collected from each participating home in 12 districts in 2010 and all nets were examined for holes and tears. One household member was surveyed about net use and care. We collected 920 polyester nets with a median age of 31 months (range: 27-44 months) and a mean of 23 (range: 0-180) holes/tears. Only 10% of the nets showed signs of repair. The mean total hole area differed between nets 36-41 months old compared to nets 30-35 months old [ANOVA, F (3, 713)=5.17, P<0.0015], but did not differ between other age groups, most notably the newest nets aged <30 months compared to those aged 42 months and older. The mean deltamethrin level was 23 mg/m^2 (<10 mg/m^2 is considered effective). LLINs used with reed mats had a higher mean number of holes/tears compared to LLINs used with commercial mattresses (two-tailed t-test, p<0.01). There were more holes/tears in the lower half of the nets (repeated measures ANOVA, F =40.74, p<0.0001) compared to other parts of the net. The finding that the oldest and newest nets had equivalent large total hole areas suggests that physical deterioration of nets occurs prior to 27 months. Nets are often tucked under reed mats which may explain the findings that nets used with reed mats had the highest number of holes and why most holes/tears were found in the lower half of the net. Studies need to be conducted prospectively starting at 3-6 months of use to determine when physical deterioration occurs. Re-enforcing the lower half of the side of each LLIN may help decrease holes/tears and LLIN users should be encouraged to repair nets.

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EFFECT OF THE DIFFERENT FACTORS ON THE DEVELOPMENT OF *PLASMODIUM* IN *ANOPHELES* MOSQUITO

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Interrupt transmission is premised on the relationship between *Plasmodium* and the mosquito-borne in-depth research. We initially investigate that different factors affect the development of *P. yoelii* in two different *Anopheles*. The effect of intestinal flora and TEP1 on the development of *P. yoelii* in *Anopheles dirus* was studied. The number of oocysts in antibiotics treated mosquitoes were significantly higher than normal infected mosquitoes. Inhibited intestinal flora made the number of oocysts increasing 7-10 fold, and infection rates nearly enhancing 1-fold. The results indicated that suppressed intestinal flora caused increasing the susceptibility of *P. yoelii* to *An. dirus*, and suggested that the intestinal flora may play an important role in the infection process of *P. yoelii* to *An. dirus*. The result of RT-PCR showed that the transcription level of TEP1 cDNA were significantly down-ragulated in the treated group.The RNAi of TEP1 increased the number of *Plasmodium* oocysts in the midgut of *An. dirus*, and enhanced the infection rate. The results indicated that the intestinal flora can not inhibit the development of parasite in *Anopheles dirus* without TEP1.The intestinal flora may affect the infection of *Plasmodium* to *An. dirus* by regulating the expression of TEP1; TEP1 may be involved in basis immune response in mosquito, which it maintain the normal intestinal flora. However, the parasite invasion caused the variation of intestinal flora that stimulated the increased expression of TEP1. The Artemether affects the development of *Plasmodium yoelii* in *Anopheles stephensi* was studied. The development of oocysts in *An. stephensi* treated by artemether were better than that of in normal *Anopheles stephensi*, and in artemether treatment group, the number of developing parasites was higher than that of in untreated mosquitoes. The results demonstrated that artemether may promote the development of *P. yoelii* in *An. stephensi*.Artemether enhance the development of *P. yoelii* in *Anopheles stephensi,* RT-PCR results showed that artemether inhibited three important *An. stephensi* immune-related genes previously described as being differentially transcribed during *Plasmodium* infection.The results suggested that artemether may weak *An. stephensi* immune response against *Plasmodiumi.*

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ASSESSMENT OF THE IMPACT OF TREATING *PLASMODIUM FALCIPARUM* ASYMPTOMATIC CARRIERS ON THE DYNAMIC OF MALARIA TRANSMISSION

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Plasmodium falciparum malaria is thought to be responsible for approximately 1 million deaths every year, mainly in children aged under 5 years. In areas that have already implemented strategies to reduce malaria transmission (e.g. distribution of insecticide-treated bed nets [ITNs],

widespread adoption of artemisinin-based combination therapy [ACT]), additional complementary interventions are required to further accelerate the reduction in disease burden. Asymptomatic carriers of *P. falciparum* serve as a reservoir of parasites for malaria transmission, and community screening and treatment of asymptomatic carriers with ACT may reduce the pool of infectious gametocytes and influence malaria transmission in that area. The description of malaria epidemiology is often focused on clinical parameters such as prevalence of parasitemia. However, entomological parameters such as vector species and density, proportion of infected mosquitoes, and the entomological inoculation rate (EIR) are essential to the understanding of the epidemiology of malaria in a specific area and the planning of control measures. The EIR is a standard measure of transmission intensity, obtained by multiplying the human-biting rate by the proportion of sporozoite-positive mosquitoes. This longitudinal survey will assess the EIR in 5 villages in Burkina Faso where mass screening and treatment of asymptomatic carriers is being implemented, and 5 control villages. In order to measure the impact of the intervention, entomological parameters will be assessed before and after the ACT treatment. During this survey, the mosquitoes will be collected once per week using the indoor spray catch method, which is a standard method for collecting indoor resting adult mosquitoes. Data collected will be used to infer the human-biting rates (the number of biting mosquitoes per human per night). Moquitoes collected will be processed by ELISA assay to estimate the sporozoite index and the EIR in each site. The population will be provided with long-lasting ITNs, and the susceptibility of malaria vectors to insecticide will also be determined. Results of this study are expected to be reported in 2012.

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DOUBLE PROOFING HOUSES AGAINST MOSQUITOES - EARLY EVALUATION

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The Roll Back Malaria campaign set a goal of reducing malaria cases by 50 % in 2010, which was not reached. Additional tools are needed than bednets that rely on compliance. We developed a prototype of double proofing houses using nets produced for bednets to make eve nets, window and door screens. According to WHOPES, such netting will remain effective for at least 3 years. Arranged between the roof and the wall, eve netting receives little sun and rain and can be expected to be effective for long time. During our pre-evaluation tests, useful experience was gained that can guide similar projects to justify reported here. Polyethylene nets or PVC covered net were fixed under the roof to prevent insects from entering the sizable eve openings or used as door hangers and window screens. The effective coverage and state of the nets were evaluated after 1 and 6 month, 1, 2 and 5 years. Nets without insecticide needed to fit tightly to be effective. Even small openings between corrugated roof and the net needed to be covered. Several ways of attaching the net were tested to identify those that were lasting, cheap, fast to install and easy to repair for the inhabitants. These trials gave valuable experience on the way mud lined houses influenced wall-related treatments. Window screens did not cause any problems and stayed in place. Door screen were more difficult to make simple. The door thresholds and floor behind are irregular, and a door screen in a frame thus leaves openings. The best arrangement was two overlapping pieces of LN netting each fixed to the upper frame and one side of the door. However, solid PE net with 4x4 mm mesh curled up within a week due to the daily pushing of the net. The more flexible Netprotect lasted a month, and even a very stiff woven agricultural insect net (no insecticide) resisted daily use less than 6 months. Best results were obtained when a load at the lower margin kept it straight, but these often fell off. Additional designs are being developed. Eve nets and simple window screens with insecticide are easy to install (1 hr for a house for 6 persons), effective in keeping insects out including mosquitoes, popular with inhabitants, and well sustained over 5 years. The cost of material is less than for 3 bednets, and the compliance problem is smaller. Since less material is involved, it will also be much cheaper than wall covering textiles. Potentially, they can be treated with other insecticides than bednets and thus be a resistant controlling tool.

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DEVELOPMENT OF A PCR-RFLP-ITS2 ASSAY TO DISCRIMINATE ITS2 GROUPS WITHIN THE *ANOPHELES TRIANNULATUS* COMPLEX (*DIPTERA: CULICIDAE*)

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Previous research has identified three *Anopheles triannulatus* species within the Triannulatus Complex: *An. triannulatus* s.s., *An. halophylus*, and *An. triannulatus* "C". As part of a larger study, 50 *An. triannulatus* s.l. samples from Argentina, Bolivia, Brazil, Colombia, Ecuador, Panama, Venezuela, and Trinidad-Tobago were sequenced for the ribosomal internal transcribed spacer 2 region (ITS2). Three separate haplotype groups were defined by a statistical parsimony network, with *An. halophylus* and *An. triannulatus* "C" in the same group. ITS2 is frequently used to discriminate species due to sequence differences, for which species-specific primers can be designed or enzyme restriction sites can be used to produce different gel banding patterns. In this study, we designed a PCR-RFLP double endonuclease restriction digest of the ITS2 region to distinguish among the three *An. triannulatus* s.l. haplotype groups. ITS2 sequences were 570-575 bp in length for group 1 (N=57; initially identified as *An. triannulatus* s.s.), 542 bp for group 2 (N=31; initially identified as *An. triannulatus* s.s.), and 560 bp for group 3 (N=11; *An. halophylus* and *An. triannulatus* "C"). These latter species were identified molecularly using a species diagnostic allozyme locus and RAPD-PCR. ITS2 length differences among groups were not easily discriminated using agarose gels. Diagnostic banding patterns were developed upon digestion of the ITS2 PCR products using the enzymes *Ale* I and *Hae* III. Our assay consistently detected three specific banding patterns for each group as follows: group 1 (275, 149, 108, 38 bp), group 2 (355, 148, 38 bp), and group 3 (415, 108, 37 bp). This assay is a rapid, inexpensive molecular method to distinguish among the three ITS2 groups within the Triannulatus Complex throughout its distribution range. Accurate identification of species within this complex is the first step to identifying its potential involvement in malaria transmission, thus impacting future vector control methods.

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TRANSCRIPTIONAL PROFILING AS AN ALTERNATIVE METHOD FOR *ANOPHELE GAMBIAE* AGE-GRADING

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Mosquito longevity or aging is an important parameter in malaria vectorial capacity, however reliable age-grading markers are lacking. Aging is associated with expression change of many genes; therefore, gene expression profiling may be used as a biomarker for age grading. We have

identified several genes that their expression monotonically changes with *Anopheles gambiae* mosquito age. This study tested the performance of expression profiling of three candidate genes in age grading. We used 2 different *An. gambiae* populations, including G3 strain in the insectary environment in the US, and Mbita strain in MalariaSphere environment, and validate the results with the mark-release-recapture method. The chronological age of these mosquitoes ranged from 1-46 days. We found that the calibration model for mosquito ageing using the mosquitoes from the insectary in the US can not be used to predict mosquito age from the field in western Kenya, however the calibration model using the mosquitoes from the MalariaSphere produce more reliable estimation of mosquito age. The study is currently being replicated in western Kenya.

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GENETIC ANALYSIS OF THE *PLASMODIUM* KILLING MECHANISM MEDIATED BY A BACTERIUM ISOLATED FROM WILD MOSQUITOES

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Following ingestion by a female *Anopheles* mosquito, *Plasmodium* parasites encounter a hostile environment of mosquito-derived factors, host blood- derived factors, and resident bacteria in the midgut lumen. Commensal bacteria in the midgut have a profound effect on the ability of the parasite to transition through discrete developmental stages before transmission to another host can occur. Studies have implicated the mosquito antibacterial innate immune response in *Plasmodium* killing. Recently, we isolated an Enterobacter bacterium from wild mosquito populations in Zambia (Esp_Z) that directly kills developing parasites in the midgut lumen and have established bacterial production of reactive oxygen molecules as the basis for inhibition both *in vitro* and *in vivo*. Using genetic and biochemical techniques, we have begun to further dissect the inhibitory mechanism exhibited by this specific bacterium. We have sequenced the genome of Esp_Z and are undertaking transcriptomic analyses to investigate both how the bacterium adapts to the mosquito midgut environment and what bacterial factors are involved in the reactive oxygen molecule-mediated inhibition of parasite development. With a better understanding of the mechanism utilized by Esp_Z or other commensal bacteria to inhibit *Plasmodium* infection of the mosquito these bacteria may eventually be introduced into wild mosquito populations as a means to curb malaria transmission.

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CHARACTERIZING THE ROLE OF *SEMA1A* IN THE DEVELOPING *AEDES AEGYPTI* LARVAL OLFACTORY SYSTEM

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Mosquito genome projects have revealed orthologs of many genes that are known to regulate development. Although characterization of the function of these mosquito genes could provide insight into the evolution of insect development and potentially reveal novel strategies for vector control, extremely little is known about mosquito development. Characterizing the development of the olfactory system is of particular interest because it is required for mosquito survival, reproduction, and blood-meal host location. The olfactory system, therefore, has both direct and indirect roles in disease transmission. We have chosen to study the developing *Aedes aegypti* larval olfactory system, which is simplified yet representative of its adult counterpart. Based on our previous work in which we analyzed the functions of the axon guidance molecule Sema1a during *Ae. aegypti* embryonic development through siRNA-mediated knockdown, we hypothesize that this molecule will function in *Ae. aegypti* olfactory system development. *sema1a* expression is localized to the brain and antennal rudiments of developing *Ae. aegypti* 2nd and 3rd instar larvae, suggesting it plays a role in the developing olfactory system. To test our hypothesis, we are studying the effects of siRNA-mediated knockdown of *sema1a* in larvae through injection of siRNA into the thoracic region of late 1st instar larvae. We have achieved significant knockdown in larvae at 24 and 48 hours post-injection and are characterizing the *sema1a* knockdown phenotype. This investigation, in combination with our ongoing functional analyses of additional developmental genes of vector importance, is helping to establish *Ae. aegypti* as an emerging model for vector mosquito development.

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IDENTIFICATION OF SPLICING REGULATORS OF HYPER-VARIABLE PATTERN RECOGNITION RECEPTOR DOWN SYNDROME CELL-ADHESION MOLECULE IN *ANOPHELES GAMBIAE*

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The vertebrate is known to generate protein diversity through alternative splicing and through this mechanism a single gene can generate multiple splice-forms. In mosquito innate immunity system, AgDscam, *Anopheles gambiae* Down syndrome cell adhesion, is an essential hypervariable receptor of the *A. gambiae* immune surveillance system which has potential to generate 31,920 alternative splice forms and produces splice form repertoires that are pathogen challenge-specific. In the previous and current parallel ongoing work we have shown that AgDscam plays important roles in defending both rodent and human malaria parasites in the mosquitoes and AgDscam's anti-*Plasmodium* responses are splice-form specific. In this study, we are focusing on identification of alternative splicing regulators of AgDscam and elucidation whether these putative splicing factors are regulated by major immune pathways. To select the candidate putative splicing factors, we first performed BLAST search based on *Drosophila* putative splicing factors against *A. gambiae* full genome and identified a panel of orthologues in *A. gambiae* for further analysis. Through RNAi-based *in-vitro* screening in the mosquito Sua5B cell line system together with expression analysis we have identified several putative splicing factors which are shown implication in the splicing of AgDscam. One of the factor identified here, AgCaper, is induced with lipopolysaccharide challenge in the Sua5B cells, while not regulated upon *Plasmodium* infection in the mosquitoes. However, RNAi gene silencing of *AgCaper* resulted in significant susceptibility of *A. gambiae* mosquitoes to *P. falciparum* infection suggests that AgCaper is implicated in anti-*Plasmodium* defense. Further thorough studies are undertaken to see how these splicing factors are regulated by the immune pathways with regarding to AgDscam alternative splicing.

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INTERBREEDING ERODES GENOMIC DIFFERENTIATION BETWEEN MOLECULAR FORMS OF *ANOPHELES GAMBIAE SENSU STRICTO* IN WEST AFRICA

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M and S molecular forms of *Anopheles gambiae sensu stricto* (*s.s.*) have been considered as marking incipient species with strong reproductive isolation in sympatry, although surveys have recently identified a few sites in the extreme west of Africa with high frequencies of naturally occurring M/S hybrid forms. Here we report new surveys of 12 sites in 4 contiguous countries (The Gambia, Senegal, Guinea Bissau, and Republic of Guinea) in 2008 and 2009, and for the Njabakunda site in The Gambia, we present monthly longitudinal data over 2 years together with a genome-wide scan for differentiation between M and S forms at this site. A total of 3499 *An. gambiae s.s.* were sampled by light trap and pyrethrum spray room collections and genotyped. High frequencies of M/S hybrid forms were seen at each site, ranging from 5% to 42%, and there was a large spectrum of inbreeding coefficient values from 0.11 to 0.76, spanning most of the possible range from zero (under panmixia) to 1.0 (if forms were genetically isolated). In Njabakunda, M/S hybrid forms were seen throughout both years including dry seasons, and differentiation between pools of homozygous M and S mosquitoes was seen only in the pericentromeric region of the X chromosome that contains the molecular form marker locus. The absence of differentiation elsewhere in the genome and high frequencies of M/S heterozygotes indicate that M and S forms are not genetically incipient species in this part of West Africa.

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A MOSQUITO LOCUS ASSOCIATED WITH GENOTYPE-BY-GENOTYPE INTERACTIONS BETWEEN DENGUE VIRUSES AND *AEDES AEGYPTI*

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Many host-pathogen systems are governed by genotype-by-genotype (G x G) interactions, whereby infection success depends on the specific combination of host and pathogen genotypes. We previously reported significant G x G interactions underlying dengue virus propagation in *Aedes aegypti* based on the comparison between three mosquito isofemale families derived from a large outbred population that had been exposed to three viral isolates. Here, we went one step further by testing associations between infection phenotype and genetic polymorphisms within the isofemale families. *Dicer-2* is a key gene of the RNA interference pathway, which functional role in the control of dengue virus infection in *Ae. aegypti* has been recently established. *Dicer-2* was polymorphic in both the outbred parental population and among siblings within the families. Allelic patterns indicated that each family derived from a single, independent mating pair and had a different recombination history at this locus. In a nested statistical analysis that included the three families, *Dicer-2* genotype was significantly associated with isolate-specific viral dissemination success, providing evidence for G x G interactions at the level of a single *Ae. aegypti* candidate gene. We also identified 172 isolate-specific single nucleotide polymorphisms in the viral genome (i.e., 1.6% of the complete genomic sequence) potentially involved in G x G interactions. Our findings indicate that the ability of dengue viruses to disseminate in *Ae. aegypti* is strongly influenced by specific combinations of *Dicer-2* genotypes and a relatively small number of viral polymorphisms, which is a starting point for functional characterization of the molecular basis underlying this specific vector-virus interaction.

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A SERINE PROTEASE INHIBITOR IN THE MOSQUITO *ANOPHELES GAMBIAE* AFFECTS VECTOR COMPETENCE FOR *PLASMODIUM* PARASITES IN THE ABSENCE OF MIDGUT BACTERIA

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Anopheles gambiae is the principle vector of the most important human malaria parasite *Plasmodium falciparum*. Infected mosquitoes mount an immune response against the parasite primarily through the Toll and IMD (immune deficiency) signaling pathways. Mosquito midgut bacteria activate these immune signaling pathways which results in anti-plasmodium effector gene expression; however no response activated specifically by the parasite in absence of bacteria has been reporte previously. To address the existence of *Plasmodium*-specific responses in *An. gambiae*, we assessed by whole-genome microarray the transcript abundance of genes in mosquitoes with their midgut bacteria removed (aseptic) through antibiotic treatment. When the transcript abundances of *Plasmodium*-infected and uninfected, aseptic mosquitoes were compared, we identified among other genes a serine protease inhibitor, serpin7, that is significantly upregulated upon parasite infection of the midgut. Silencing of the serpin7 transcript results in a significant reduction in the numbers of both *P. falciparum* and *P. berghei* (rodent parasite) oocysts. Serpin7 does not appear to have an effect on the expression of several Toll and IMD immune effector genes with known anti-*Plasmodium* activity, suggest that serpin7 is not a regulator of these pathways. We are currently addressing the hypothesis that serpin 7 is part of a bacteria -independent anti-*Plasmodium* defense system.

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COMPLEXITY OF GENETIC VARIATIONS IN WILD *ANOPHELES GAMBIAE* POPULATION IN KENYA

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Malaria causes millions of deaths every year. It is transmitted by anopheline mosquitoes, among which *Anopheles gambiae* is the dominant human malaria vector. Mosquitoes that are resistant and susceptible to malaria have been observed in *A. gambiae* population at malaria endemic Kenya. The genetic variations are believed to be responsible for the malaria resistance in *A. gambiae* mosquitoes. This study focuses on the genetic variations in wild *A. gambiae* population in Kenya. We collected wild *A. gambiae* mosquitoes, and sequenced ~20 individual mosquito genomes using Illumina technology. We analyzed the short reads to obtain single nucleotide polymorphisms (SNPs) and gene structure variations. The variations are being analyzed to get haplotypes, so that we can calculate the linkage disequilibrium size in *A. gambiae* population at Kenya. We found roughly one million SNPs, ~90% of which do not overlap with any known SNPs. Consistent to our previous findings, many transcript structure variations contain SNP at processing sites, indicating that these transcript variations are from allelic gene structure variations. Since the genetic

information for each locus is multi-dimensional, we have developed databases and visualization tools with the integration of gbrowse to display the gene structures, alternative splicing, protein functions, SNP, LD, sequence reads, and EST. The genetic variations obtained in this project will provide a new research foundation for mosquito-malaria and mosquito-insecticide interaction, which may inform novel malaria control strategy.

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COMPARATIVE TRANSCRIPTOMICS OF THREE *AEDES AEGYPTI* STRAINS

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RNA-seq technology allows unprecedented levels of transcriptome analyses by deriving the transcriptional map of an organism, tissue or cell at predefined conditions. Given a group of organisms that differ in a particular phenotype, comparisons of the expression profiles of such groups will provide information on the networks of genes, their transcriptional regulation and their effect on the phenotypes under analyses. RNA-seq technology was used to compare the transcriptomes of three strains of *Aedes aegypti* with different susceptibility to dengue virus infection. This mosquito is the main vector of all four dengue serotypes (DENVs), the Yellow fever virus (YF) and Chikungunya virus (CV). In each case, viral particles are acquired by the mosquito via blood feeding on an infected human and the virus is transmitted to another human host through a subsequent blood meal. Therefore, the efficiency of virus infectivity within the mosquitoes and its transmission is associated closely with blood ingestion and digestion. However, geographically distinct *Ae. aegypti* populations show different vector competence. Comparisons of differences in the transcriptome before and after a blood meal among strains of *Ae. aegypti* revealed aspects of phenotypic plasticity that may correlate with the different vectorial capacities of each strain. Additionally, putative cis regulatory elements (CRE) were identified that may be responsible for coordinate gene regulation following the blood meal. CREs are essential components of proposed genetic-based vector control strategies whereby mosquito populations are suppressed in a sex-specific manner or modified by introgressing anti-pathogen effector genes.

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USE OF A CULICINE MOSQUITO PROMOTER TO INDUCE FEMALE-SPECIFIC GENE ACTIVITY IN *ANOPHELES STEPHENSI* LARVAE

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Malaria, vectored by female anopheline mosquitoes, continues to pose a serious threat to human health worldwide and requires novel solutions for control of both the disease vector and pathogen transmission. Novel mosquito control strategies involving transgenesis to modify vector populations depend on well-characterized gene regulatory modules. We have functionally characterized the promoter and a fat body- and female-specific enhancer from a culicine hexamerin gene (*Hexamerin-1.2*). Using transgenic lines made in a distantly related fruit fly, *Drosophila melanogaster*, we have shown that a short *Hex* enhancer can work with a *Drosophila* promoter to drive activity of the *lacZ* reporter gene exclusively in the fat body tissue of late-stage female larvae and young female adults. We also determined that within the *Hex* enhancer three specific binding sites for the highly conserved transcription factor, Doublesex (DSX), are necessary to maintain female-biased reporter gene activity in *Drosophila*. However, the same enhancer/promoter combination (including the DSX sites) can elicit true female-specific reporter expression in one of three transgenic lines of a related culicine mosquito, *Aedes aegypti.* Hence, in a related mosquito, chromosomal position may determine if the *Hex* enhancer drives exclusive female-specific gene activity. We are currently testing this culicine hexamerin gene enhancer and promoter in the more distantly related mosquito, *Anopheles stephensi,* to determine if the DSX sites of the *Hex* enhancer can also override specific chromosomal position effects in anopheline mosquitoes. No transgenic technology can currently act as a female-specific mosquito larvicide. Inducing true female-specific gene activity in immature anopheline mosquitoes through the *Hex-1.2* promoter and enhancer should allow the development of a number of novel mosquito control strategies, including genetic sexing strains.

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DRY SEASON'S DETERMINANTS OF MALARIA DISEASE AND NET USE IN BENIN, WEST AFRICA

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To achieve malaria eradication, vector control efforts will have to be sustained even when the incidence of malaria cases becomes low. In this work, malaria incidence was evaluated in children of under 5 years of age in 28 villages in Benin during the dry season. Using mixed-effect models, malaria incidence was assessed according to the level of transmission by different vector species, and LLIN use and ownership. Then, a logistic mixed-effect model was developed to assess whether night-time temperature, biting nuisance and LLIN ownership are good predictors of "effective" LLIN use. Results showed that *Anopheles funestus* rather than *An. gambiae* s.s. is responsible for malaria transmission. A rate of LLIN use <60% was associated with a higher risk of malaria, and nocturnal temperature and biting nuisance were predictors of effective LLIN use. This study emphasized the need for a better understanding of the epidemiology of malaria during the dry season.

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MOSQUITO SURVEY OF ST. KITTS AND NEVIS

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Classic mosquito surveillance studies in the Federation of Saint Kitts and Nevis (SKN) predate the major range expansion of *Aedes albopictus.* To update these studies and determine whether any novel species have been introduced, a mosquito survey was performed. Surveys were performed in the dry season (Mar. 16-23, 2010) in St. Kitts and repeated in the rainy season (Oct. 18-25, 2010) in SKN. BG Sentinel traps were set with CO_2 in a variety of habitats (urban, rural, mangrove, dry forest). Identification was performed using morphological keys, and RT-PCR testing for dengue, West Nile and chikungunya viruses is pending. In the dry season, 4,279 mosquitoes were trapped in 73 trap periods. In the rainy season, 2,626 mosquitoes were trapped in St Kitts in 56 trap periods. The most abundant species during both phases were *Culex quinquefasciatus* (68% dry, 40% rainy), *Ae. taeniorhynchus* (19%, 42%), and *Ae. aegypti* (8%, 11%). Urban and semi-urban sites mainly yielded *Cx. quinquefasciatus* and *Ae. aegypti*, while those near mangroves yielded more species diversity. There were 3 new records for St. Kitts: *Anopheles albimanus*, *Culex nigripalpus* and *Ae. tortilis*. Traps were set for five trap periods in Nevis and 659 mosquitoes were collected. This is the first time a potential malaria vector has been identified from St. Kitts. Other potential disease vectors, *Cx. quinquefasciatus* and *Ae. aegypti*, were found throughout SKN. No *Ae. albopictus* were found during this survey.

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LARVAL HABITAT SUCCESSION FOR *ANOPHELES GAMBIAE* S.L IN DIFFERENT LAND USE TYPES IN WESTERN KENYA HIGHLANDS

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Knowledge of habitat succession of *Anopheles gambiae* s.l mosquitoes is important to the understanding of mosquito population regulation and malaria transmission. This study investigated the *An.gambiae* s.l larval habitats succession in western Kenya highlands to understand the parameters influencing the mosquito species abundance in different habitats and land use management practices. Longitudinal study was conducted to ascertain the abundance and succession of *An.gambiae* s.l larvae in different habitats and land use types for two years. Larvae sampling and physical chemical analysis of water were done weekly. Habitats size, chlorophyll a and grass cover were monitored. It was found that, grass cover, chlorophyll a, nitrates, phosphates and habitat surface area had correlations with species abundance and succession. Land use types had influence on habitat larvae abundance. The correlation of variables differs from habitat types and land use types. Knowledge of larval habitat succession and seasonal abundance of *An.gambiae* s.l enhances the cost effective malaria vector control programme planning.

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INVESTIGATING THE RELATIONSHIP BETWEEN DIFFERENT LEVELS OF ITN USAGE AND THE BITING PATTERN OF *ANOPHELES* MOSQUITOES IN NORTHERN GHANA

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Anopheles gambiae and *An. funestus* remain the major species that transmit malaria and Lymphatic filariasis in Ghana. The major vector control strategy of these species has been the use of insecticides treated bed nets (ITNs). Previous research work in Kassena-Nankana district (KND) in the Upper East region an area with high ITN usage showed a change in the biting pattern of *Anopheles* species where another biting peak was observed in the early hours of 5am-6am in addition to the normal peak of 12am-2am. This was attributed to the extensive use of ITNs in the area. In the Bongo district another area located in the Upper East region, ITN usage is very low. This study was to determine the biting pattern of *Anopheles* mosquitoes in the area of low ITN usage. Adult mosquitoes were collected in the rainy season using human landing catches from the hours of 18:00 to 06:00 and pyrethroid spray catches (PSC) over a period of five months. *An. gambiae* and *An. funestus* were identified as the major mosquito species in the area. The biting pattern of *An. gambiae* showed two peaks (12-2am and 4-6am) in the early hours of the morning as was observed in KND with high ITN usage. *An. funestus* however, showed a continuous rise in biting rate up to the early hours of the morning. Sporozoite infectivity of the major vectors in the area was 3.9% (n = 542). This change in biting pattern has implications on malaria/LF transmissions since hosts are less protected during these early hours. However, the study showed that there is no direct correlation between level of ITN usage and the change in biting pattern of mosquitoes since a similar trend of biting pattern was observed in Bongo district and KND. The change in biting pattern may be due to other extrinsic factors that may affect the behavior of the mosquitoes in the area. The implications of these findings in the context of malaria/LF transmission and control will be discussed.

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HUMAN IGG ANTIBODY RESPONSE TO SALIVARY NTERM-34KDA PEPTIDE AS AN IMMUNO-EPIDEMIOLOGICAL BIOMARKER FOR EVALUATING THE EXPOSURE TO *AEDES AEGYPTI,* A MAJOR VECTOR OF HUMAN ARBOVIRUSES?

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Measuring human exposure to arthropod bites, would allow to evaluate the transmission risk of some vector-borne diseases. *Aedes aegypti* (Diptera: Culicidae) is involved in the transmission of arboviruses such as dengue, Chikungunya and yellow fever. The present study aim at evaluating human specific antibody (Ab) IgG response to a salivary candidate peptide (the Nterm-34kDa peptide) specific to *Ae. aegypti*, and its potential use in measuring exposure to *Ae. aegypti* bites. A longitudinal study, concerning children (n=205, aged from 0 to 5 years) living in an area where *Ae. aegypti* is endemic in southern Benin (West Africa), was performed between 2008 and 2009. For each child, the specific immune response against the Nterm-34kDa peptide was evaluated by ELISA method, from a dried blood spot on filter paper collected every 6 weeks. The results demonstrated for the first time, the existence of a specific IgG Ab response to Nterm-34kDa peptide in children exposed to *Aedes aegypti* bites. A significant increase of this specific response was observed from the dry season (period of low exposure to *Aedes* bites) to the rainy season (period of high exposure to *Aedes* bites). In addition the IgG Ab level presented spatial heterogeneity between individuals and between the studied villages. Finally, age and sex seemed to have no influence in the level of the anti-Nterm-34kDa peptide IgG Ab. This preliminary study showed that IgG Ab response to the candidate Nterm-34 kDa could be a promising alternative for evaluating the level of exposure to *Aedes aegypti* and then the potential risk for getting arboviruses, or the efficacy of vector control campaigns against this mosquito. It will be essential to validate this immuno-epidemiological tool by conducting further studies taking into account entomological measurements.

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TOPOGRAPHY AS A MODIFIER OF BREEDING HABITATS AND CONSEQUENTLY VULNERABILITY TO MALARIA IN THE WESTERN KENYA HIGHLANDS

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Topographic parameters such as elevation, slope, aspect, and ruggedness play an important role in malaria transmission in the highland areas. They affect biological systems, such as larval habitats presence and productivity for malaria mosquitoes. This study investigated whether local spatial malaria vectors distribution and risk of infection with malaria parasite in the highlands is related to topography. Four villages each measuring 9Km^2 lying between 1400-1700m above sea level in western Kenya highlands were categorized into a pair of broad and narrow valley shaped terrain sites. Larval, Indoor resting adult malaria vectors and infection surveys were collected originating from the valley bottom and ending at the hilltop both sides of the valley during the rainy and the dry season. Data collected a distance of ≤500m from the main river/stream were categorized as valley bottom and those above as uphill. Larval surveys were categorized by

habitat location while vectors and infections by house location. In overall, broad flat bottomed valleys had significantly high number of anopheles larvae per habitat than narrow valleys both during the dry (1.60 versus 1.18 larval/habitat) and the rainy seasons (3.09 versus 1.60 larval/habitat). Similarly, vector adult densities/house in broad valley villages was higher than those within narrow valley houses during both the dry (0.64 versus 0.33) and the rainy season (0.80 versus 0.08). Asymptomatic malaria prevalence was significantly higher in participants residing within broad valley villages than those in narrow valley villages during the dry (14.6% vs. 7.8%) and rainy (16.7% vs 1.2%) season. Clinical malaria cases were wide spread in both valley and uphill villages in broad valley villages during both the dry and rainy season, whereas over 65% of infections were clustered at the valley bottom in narrow valley villages during both seasons. In conclusion, despite being in the highlands, local areas within low gradient topography characterized by broad valley bottoms have stable and significantly high malaria risk unlike those with steep gradient topography which exhibit seasonal variations. Topographic parameters could therefore be considered in identification of high-risk malaria foci to help enhance surveillance or targeted control activities in regions where they are most needed.

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THE SPATIAL AND TEMPORAL DYNAMICS OF DENGUE IN SOUTHERN VIETNAM

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Dengue is a major public health problem in southern Vietnam. Dengue transmission dynamics, which comprise of annual and multi-annual cycles, are complex and poorly understood. Previously, we studied dengue transmission in one province and hypothesized that epidemics emanate from larger cities (e.g. Ho Chi Minh City) in southern Vietnam. In this study, dengue reported incidence data of 178 districts of 20 provinces from 2001 to 2010 in southern Vietnam were characterized and further analyzed to unravel its transmission dynamics and spatio-temporal patterns. Wavelet analyses were performed on time series of Province-level monthly notified dengue cases (i) to determine the periodicity dengue incidence, (ii) to analyze synchronicity between the districts and provinces, (iii) to characterize the spatial-temporal relationships between districts and larger cities, and (v) to associate the relationship between dengue incidence and local climate. A continuous annual mode of oscillation and a multi-annual cycle of around 2-3-years were observed from 2003-2008 in Ho Chi Minh City and the majority of provinces. Synchrony in time and space in both the annual and 2-3-year cycle were observed. Phase differences were used to describe the spatio-temporal patterns, which suggested that the annual wave of infection was either synchronous among western provinces or moving away from Ho Chi Minh city. Inversely, the 2-3-year periodic wave was moving towards, rather than away from Ho Chi Minh City. A strong non-stationary association between climate variables with dengue incidence in the 2-3-year periodic band was found. In conclusion, collectively, analyses on dengue incidence of 20 provinces from southern Vietnam confirmed multi-annual cycles of dengue transmission. In contrast to Thailand, spatio-temporal revealed that epidemics in Vietnam emanate from provinces, rather from larger cities (e.g. Ho Chi Minh City).

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PRODUCTIVITY OF MALARIA VECTORS FROM DIFFERENT HABITAT TYPES IN THE WESTERN KENYA HIGHLANDS

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Mosquito Larval Source Management (LSM) could be a valuable additional tool for integrated malaria vector control especially in areas with focal transmission like the highlands of western Kenya if it were not for the need to target all potential habitats at frequent intervals. The ability to determine the productivity of malaria vectors from identified habitats might be used to target LSM only at productive ones. Each aquatic habitat within three highland sites in western Kenya was classified as natural swamp, cultivated swamp, river fringe, puddle, open drain or burrow pit. Three habitats of each type were selected in each site in order to study the weekly productivity of adult malaria vectors from February to May 2009 using a sweep-net and their habitat characteristics recorded. All surveyed habitat types produced adult malaria vectors. Mean adult productivity of *Anopheles gambiae* sensu lato in puddles (1.8/m2) was 11 - 900 times higher than in the other habitat types. However, puddles were the most unstable habitats having water at 43% of all sampling occasions and accounted for 5% of all habitats mapped in the study areas whereas open drains accounted for 72%. Densities of anopheline late instars larvae significantly increased with the presence of a biofilm but decreased with increasing surface area or when water was flowing. Taking stability and frequency of the habitat into account, puddles were still the most productive habitat types for malaria vectors but closely followed by open drains. In conclusion, even though productivity of *An. gambiae* s.l. was greatest in small and unstable habitats, estimation of their overall productivity in an area needs to consider the more stable habitats over time and their surface extension. Therefore, targeting only the highly productive habitats is unlikely to provide sufficient reduction in malaria vector densities.

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SPATIAL AND TEMPORAL EVALUATION OF *AEDES AEGYPTI* BREEDING SITES IN BELLO, COLOMBIA

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Immature stages of *Aedes aegyti* have been used to calculate several "entomological indices" of abundance of dengue vectors; some studies have concluded that these indices can be used as indicators of risk of dengue epidemics, while other studies have failed to find a predictive relationship. Ecological niche models have shown ability to predict distributional patterns in space and time, not only of vectors, but also of the diseases that they transmit. In this study, we used Landsat 7 ETM+ images and two niche-modeling algorithms to estimate the local-landscape ecological niche of *Ae. aegypti* breeding in Bello, Colombia, and to evaluate its potential spatial and temporal distribution. Our models showed low omission error indices with high confidence levels: about 13.4% of the area presents conditions consistently suitable for breeding across the entire study period (2002-2008). The proportion of area predicted as suitable showed a weak positive association with dengue case rates by neighborhood, while the entomological indices had no relationship with the entomological indices.

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FUNGAL PATHOGEN DYNAMICS WITHIN THE DENGUE VECTOR *AEDES AEGYPTI*: AN ASSESSMENT OF FUNGAL INFECTIONS ON FEEDING BEHAVIOUR AND INTERACTIONS WITH PROTECTIVE SYMBIONTS

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The continued global impact of dengue and dengue hemorrhagic fever as an emerging infectious disease, in combination with rising insecticide resistance in the primary mosquito vector *Aedes aegypti*, indicates an urgent need for novel methods of dengue vector control. Entomopathogenic fungi such as *Beauveria bassiana* offer promise as potential biological control agents; however, little is understood about pathogen dynamics of these fungi within the mosquito host, what affect fungal infections have on behaviour and if bacterial symbionts can protect against fungal infections. We measured fungal loads of *B. bassiana* isolate FI-277, known to reduce longevity and blood-feeding success of *Ae. aegypti*, using quantitative PCR, in order to demonstrate the potential of this technique to answer a range of research questions. We found that fungal load increases slightly with time starting at day 7 post-infection after experiencing an initial trough, consistent with infection patterns reported in *Anopheles gambiae* and *Drosophila.* Additionally, fungus-infected mosquitoes that did not feed when offered a human arm for 10 minutes had higher fungus loads than fungus-infected mosquitoes that fed, indicated a relationship between pathogen load and behaviour. Finally, while the endosymbiotic bacteria *Wolbachia* had no detectable effect on mosquito survival when co-infected with *B. bassiana*, we found that *Wolbachia* infected mosquitoes had lower fungus loads than *Wolbachia*-free, isogenic control mosquitoes, suggesting an effect of *Wolbachia* on mosquito innate immunity. These results, aside from demonstrating the potential of *B. bassiana* as a mosquito control agent that can reduce blood-feeding success and survival even in the presence of protective symbionts, also highlight the sensitivity of quantitative PCR methods for the study of pathogen dynamics within dengue vectors, which may expand research opportunities for biological control of dengue vectors.

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ANTIGENICITY CHANGES OF SALIVARY PROTEINS AND ANTIBODY PERSISTENCE AGAINST *ANOPHELES ALBIMANUS* AND *AEDES AEGYPTI* PRINCIPAL VECTORS OF DISEASES IN COLOMBIA

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Mosquito saliva contains very active compound that are able to induce immune response. The association between the level of antibodies against vector saliva and risk of disease has been study of disease like malaria and leishmaniasis but very few in viral diseases like dengue fever. In this study we evaluated the persistence of antibodies in serum from people living in an area free of dengue and malaria transmission but surrounded by dengue endemic areas. We followed the individuals during 6 months, before and after returning from dengue endemic areas; the first cohort (n=30) was studied 2007 and the second cohort (n=45) was studied in 2010. We found a 2-fold decrease in the level of IgG antibodies anti-*Ae. aegypti* salivary gland extract and in IgG and IgM anti-*An. albimanus* saliva antibodies in a 6 month period. Interestingly, the level of IgM antibodies anti-*Ae. aegypti* did not show a significant decrease during the follow up and we think this is due to the presence of species other than *Ae. aegypti* (i.e. *Aedes* sp.) in the area. We also found that the level IgG of antibodies was higher in healthy people that have suffered malaria or dengue, or both after 4 months of living in a non-endemic area. However, this level was similar for both groups immediately after returning from travel. Western-blot results showed difference in the proteins recognized by the pool of serum on each point of the follow-up.

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DO LONG LASTING INSECTICIDAL NETS (LLINS) SUCCESSFULLY CONTROL *ANOPHELES ARABIENSIS*?

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High coverage of conventional and long-lasting insecticide treated nets (ITNs and LLINs) in parts of East Africa are associated with reductions in local malaria burdens. Shifts in the malaria vector species ratio have coincided with the scale-up suggesting that some species are being controlled by treated nets while others are not. Between 2005-2006 six experimental hut trials of ITNs and LLINs were conducted in parallel at two field stations in northeastern Tanzania, the first station in Lower Moshi Rice Irrigation Zone, an area of *Anopheles arabiensis*, and the second in coastal Muheza where *An. gambiae* and *An. funestus* predominate. Five pyrethroids and one carbamate insecticides were evaluated on the nets which were assessed in terms of insecticide-induced mortality, blood-feeding inhibition and exiting. In the experimental hut trials, the mortality of *An. arabiensis* was consistently lower than that of *An. gambiae* and *An. funestus*. The percentage mortality rates in trials with pyrethroid mosquito nets ranged from 25-52% for *An. arabiensis*, 63-88% for *An. gambiae* s.s. and 53-78% for *An. funestus*. All pyrethroid-treated nets provided considerable protection for the occupants, despite being deliberately holed, with the percentage blood-feeding inhibition not differing between the three species. Percentage mortality of *An. gambiae* and *An. arabiensis* in cone bioassays on the netting was consistent between locations. LLINs and ITNs treated with pyrethroids were more effective at killing *An. gambiae* and *An. funestus* than *An. arabiensis*. This could be a major contributing factor to the species shifts observed in East Africa following the scale up of LLINs. *An. arabiensis* females whose feeding is frustrated by the net barrier may be forced to seek hosts the next evening before people retire to beds resulting in early evening transmission. With continued expansion of LLIN coverage in Africa *An. arabiensis* is likely to remain responsible for residual malaria transmission. Supplementary control measures to LLINs may be necessary to control this vector species.

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TRACKING LONG LASTING INSECTICIDAL NETS (LLINS) DISTRIBUTED VIA NATIONAL CAMPAIGN: ASSESSING LONG LASTING INSECTICIDAL MOSQUITO NETS (LLIN) LOSS, PHYSICAL DETERIORATION, AND INSECTICIDAL DECAY IN BENIN

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The malaria vector control plan retained in the malaria control strategy of African countries for 2010 is to move from targeted distribution to universal coverage of Long Lasting Insecticidal Mosquito Nets (LLINs). This 'sustaining high coverage' objective places greater emphasis on the need for timely replacement of LLINs that are either 'lost', e.g. given away, burnt, torn up etc, and thrown out, or that fail due to insecticidal decay over limited time. To increase the accuracy with which a program

can answer the 'replacement' question, it becomes important to better understand the concept of 'LLIN effective life', and how it informs the key logistical and operational decisions related to LLIN replacement campaigns. To meet this goal, 50 PermaNet® distributed in 3 different sites from 2006 to 2009 in southern Benin were prospected and collected in each study site. The physical aspect of each net was assessed. Collection activities were reinforced by sociological investigation forms (Questionnaires) in order to figure out the history of each net. The residue of insecticides collected from the nets on Whatman#1 fiber disks were analyzed using the rapid colorimetric net test (technology CDC/Atlanta). To confirm results from the colorimetric test, cone tests were performed on a portion of ineffective nets Results of this study showed that a total of 35.5% of nets observed were found dirty. Considering torn out LLINs, 14.5% were repaired by the owners. 42.7% of holed PermaNet had more than 5 holes per face or side. After performing colorimetric test, only 21.6% (45/208) of PermaNet® had scored beyond the threshold of 0.7µg/disk (2.8µg/ deltamethrin/ m^2). Among PermaNets that had been washed, more than 5 and 10 times, the quantity of deltamethrin per net was very low and only 8.9% But after more than 10 washings, all nets had lost their efficacy. The colorimetric test developed by CDC Atlanta is a rapid method to assess the decay of insecticides in LLINs.

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INVASION DYNAMICS OF CHIKUNGUNYA VECTOR, *AEDES ALBOPICTUS* ON MAYOTTE

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In 2005-2006, a Chikungunya epidemic raged in Mayotte an island of Southwestern Indian Ocean, affecting 38% of the population (Cire Mayotte 2007) raising the problem of poor knowledge of vectors ecology there. The presence of *Aedes albopictus* on the island has played an important role in emergence of this infectious disease. This mosquito has been established on the island principally in urban area, leading to displacement of *Ae. aegypti* population in rural area only 6 years after its first description (2001). Here we undertook a comparative study between 2007 and 2010 in different types of urban landscape in order to document invasion dynamics of *Ae. albopictus*. We sought to identify urban landscape characteristics (landcover, human density, human practices) that could lead to this species infestation and to different relative abundance of immature stages of both species using a grid adapted to individual house scale. The relative proportion of *Ae. albopictus* significantly increased between both years and this species is dominant in all areas in 2010 even in less urbanized habitats. Proportion of habitats with only *Ae. albopictus* significantly increased between 2007 and 2010 either in urbanized and in rural areas whereas *Ae. aegypti* was almost never found alone in breeding sites in 2010. Proportion of non vegetated areas, the distance to closest natural zone, the inhabitants densities and the amount of wastes recorded were explicative factors of highest abundance of *Ae. albopictus*. A critical analysis of the original method used here and a first explicative model of relative abundance of these species in Mayotte are proposed. These findings are of great implication for vector control strategies and also for forecasting arboviroses outcome on such an island in the way of status changing.

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ANOPHELES FUNESTUS IN THE SENEGAL RIVER BASIN: BIONOMICS, ROLE IN MALARIA TRANSMISSION AND INSECTICIDE SUSCEPTIBILITY STATUS

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Anopheles funestus is one of the major malaria vectors in Africa. In Senegal, this species has recolonized the Senegal River basin after 30 years of absence following the implementation of two dams. Following entomological survey carried out in the area revealed that *An. funestus* is lowly involved in malaria transmission. However, the infection rate increased from 0.04 % in 2004 to 5.07 % in 2006 in the Guiers lake area. A correlated increase of parasite prevalence in children under 10 years was also reported in this part of the Senegal River Basin. It is in this context that we undertook our study to (a) update the distribution range of *An. funestus* populations in the Senegal River basin, (b) assess its role in malaria transmission and (c) determine its susceptibility status to insecticides. Prospection carried out in different parts of Senegal River Basin revealed that the main area where *An. funestus* is currently present is the south of Guiers Lake area and the nearby localities of low valley of Ferlo. Entomological survey carried out in two villages (Gankette Balla and Mbilor) revealed a low infection rate (1/1850) for *An. funestus* in Gankette Balla. The entomological inoculation rate was estimated to 2.55 infected bites for a study period of 6 months. Insecticide susceptibility assays were carried out using 2-5 day old F1 adults obtained through field collections of blood-fed females and larval rearing of *An. funestus* from Gankette Balla. WHO susceptibility test revealed that *An. funestus* is fully susceptible to fenitrothion, deltamethrin, and lambdacyalothrine with 100% mortality observed 24 h after an exposure of 1h to each of these insecticides. However suspected DDT and Dieldrin resistance was observed with respectively 88.2% and 85.3% mortality. A slight tolerance was also observed to Bendiocarb and Permethrin with respectively 93.7% and 95.8% mortality. Our study revealed a low level of malaria transmission due to *An. funestus* and a moderate resistance level to some insecticides that should be taken into account by future control programs.

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SEASONAL CHANGES IN FEEDING AND REPRODUCTION OF *ANOPHELES GAMBIAE* AS MECHANISMS FACILITATING AESTIVATION IN THE SAHEL

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Malaria remains a top public health priority in Sub-Saharan Africa, where it is transmitted primarily by *Anopheles gambiae s.l.* Populations of these cryptic species exploit diverse environments, including dry regions where surface waters required for larval development are absent for 3-7 months. Recently we have demonstrated that the M molecular form of *An. gambiae* can survive throughout the 7-month-long dry season in the Sahel (presumably via aestivation). However, the physiological and behavioral mechanisms that facilitate aestivation are as-yet unknown. We hypothesize that extended survival during aestivation is achieved based on reduced blood feeding and all other reproductive parameters. To test this hypothesis, we evaluated seasonal variation in the following parameters

of populations of *An. gambiae* in the Sahel: (i) blood-seeking response, (ii) sugar-seeking response, (iii) egg development, (iv) oviposition response, and (v) egg-batch size from October 2009 through August 2010. Data analysis is currently ongoing, however, preliminary results suggest seasonal variation in both feeding responses measured. During the pre-aestivation period (October-November), the M form showed a reduced aptitude of females for blood feeding and of males for sugar feeding. During the dry season, however, the feeding response increased and was similar to during the wet season. A comprehensive analysis of all these parameters will be presented and discussed in relation to the dry-season ecology of *An. gambiae*.

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SEARCHING FOR INVISIBLE MOSQUITOES: WHERE DOES *ANOPHELES GAMBIAE* SHELTER DURING THE DRY SEASON IN THE SAHEL?

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Malaria remains a top public health priority in Sub-Saharan Africa, where it is transmitted primarily by *Anopheles gambiae s.l.* Populations of these cryptic species exploit diverse environments including dry savannahs and semi-desert areas, where surface waters required for larval development are absent for a large part of the year. Recently we have demonstrated that the M molecular form of *A. gambiae* can survive throughout the 7-month-long dry season in the Sahel (aestivation). However, the nature of the shelter(s) used by aestivating mosquitoes is unknown. To address this question, we compared the spatial distributions of mosquitoes between the dry and wet seasons to assess if the mosquitoes are more clustered during the dry season, and if so, if the high-density houses are the same across seasons. We analyzed the relationship between various house characteristics and mosquito density during the dry season. Additionally, we used entry traps to determine if mosquitoes shelter indoors or outdoors and also sampled other putative shelters. Our results show that mosquito distribution is considerably more clustered during the dry season, than in the wet season. High density houses were clustered in a "hot zone" at one edge of the village. Moreover, during the dry season the "hot zone" was distinct from the area with high density houses during the preceding wet season. Attributes of the houses and their immediate vicinity did not explain mosquito density. Entry traps revealed that mosquitoes shelter outdoors rather than indoors. However, extensive surveys of wells, rodent burrows, termite mounds, and toilet pits were all negative for *A. gambiae* (although approximately 100 *Culex quinquefasciatus* and 1 *An. rufipes* were found). These results demonstrate that *An. gambiae* shelters outdoors, in as-yet unknown sites. Because the area where the majority of mosquitoes clustered was rather confined, the shelters are probably located within a few hundred meters of the village. Finding such shelters will be of importance for malaria control in arid areas and for understanding seasonality in mosquitoes and malaria transmission.

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KOUTANGO VIRUS INFECTION DYNAMICS IN *AEDES AEGYPTI*

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Koutango virus is a Flavivirus from the family Togaviridae and has been shown to cause a mild disease in humans. Koutango is transmitted to humans by mosquitoes, particularly Aedes spp. During a recent serosurvey of patients with acute febrile illness in Western Africa, we detected a high prevalence of Koutango exposure in people. To better characterize the potential for this emerging virus to be transmitted in the same urban cycle as dengue and yellow fever (which were also detected in the serosurvey), we undertook vector competence testing with strain DAK ArD 5443 which was isolated from Senegal in 1968. *Aedes aegypti* (Rockefeller) were orally exposed to a bloodmeal containing Koutango virus of 109 pfu/ml and subsequently tested for both abdomen infection and leg dissemination on days 3, 5, 7, and 11 post exposure. We found significant differences among these time points in both the infection and dissemination rates. Specifically, the infection and dissemination rates at days post exposure 3 (.089,.02), 5 (.32,.08), 7 (.24,.17) were significantly different from day 11 post exposure (.83,.58). These data confirm that *Ae. aegypti* is a competent vector for Koutango virus and the transmission potential of this virus is complex, owing to the early- though slight- dissemination rates and the relatively high dissemination rates at 11 days post exposure. These data can be further used to evaluate the cumulative vectorial capacity of Koutango in *Ae. aegypti* as a measure of its overall transmission potential and better inform future surveillance efforts for this emerging pathogen.

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MALARIA ENTOMOLOGICAL INOCULATION RATES IN THREE LOCALITIES OF ANTIOQUIA AND CORDOBA DEPARTMENTS IN COLOMBIA

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In Colombia, Antioquia and Cordoba departments traditionally have had the highest reports of malaria cases, accounting for a total of 67,670 (58.18%) in 2010. The characterization of entomological parameters allows for a better understanding of malaria transmission dynamics. Our objective was to evaluate entomological parameters such as abundance, biting behavior, human biting rate (HBR), infectivity rate (IR) and entomological inoculation rate (EIR) for anopheline species in three localities: La Capilla-CAP in Antioquia; and El Loro-LOR and Juan Jose-JUJ in Cordoba. Mosquito collections in each locality were performed four times, every three months, for five days, from 18:00-24:00 h and one day from 18:00-06:00 h, from November 2008-June 2010. A total of 5,320 anophelines belonging to seven species were identified. *Anopheles nuneztovari* s.l. and *An. darlingi* were the most abundant species, 69.72% and 22.01%, respectively. *An.triannulatus* s.l., *An.pseudopunctipennis*, *An.albitarsis* s.l., *An.argyritarsis* and *An.punctimacula* together accounted for 8.27%. HBR varied greatly among the different species, *An. nuneztovari* s.l. and *An. darlingi* showed biting activity throughout the night with the highest peak between 21:00-23:00-20:00-23:00h, respectively. *An. nuneztovari* s.l. exhibited an endophagic preference in LOR and CAP (t= 2.27 P< 0.05 n= 24 and t= 2.58 P< 0.05 n=23, respectively). In JUJ *An. nuneztovari* s.l. was infected with *Plasmodium falciparum* and *P. vivax* (IR%= 0.09 and 0.22, respectively). In CAP *An. nuneztovari* s.l., *An. darlingi* and *An. triannulatus* s.l. were infected with *P. vivax* (IR%= 0.01, 0.09 and 1.22, respectively). *An. nuneztovari* s.l. showed the highest EIR in JUJ (24.9 infective bites/yr), followed by *An. triannulatus* s.l. and *An. darlingi (*3.87 and 3.84, respectively). HBR and EIR results confirmed that *An. nuneztovari* s.l. and *An. darlingi* continue to play an important role in parasite transmission in these localities and suggest that *An. triannulatus* s.l. may be a local vector in this region.

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GENETIC DIVERSITY OF *ANOPHELES TRIANNULATUS* S.L. FROM LOCALITIES OF NORTHWESTERN AND SOUTHEASTERN COLOMBIA AND DETECTION OF NATURAL INFECTION BY *PLASMODIUM* SPP.

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Anopheles triannulatus s.l. is a complex consisting of at least three sibling species, *An. triannulatus* s.s, *An. halophylus* y *An. triannulatus* "C". In Colombia, it is not known whether different members of the complex are present and if they contribute to malaria transmission. This study evaluated genetic variability of specimens of *An. triannulatus* s.l. from NW and SE Colombia by PCR-RFLP-ITS2 and cytochrome oxidase subunit I (*COI)* gene sequences and determined their natural infectivity with *Plasmodium* spp. by ELISA and nested PCR. A total of 511 *An. triannulatus* s.l. were collected between January 2008 and October 2010, in the NW localities of El Bagre (BAG), Zaragoza (ZAR), San Pedro de Uraba (SPU) and Puerto Libertador (PLT) and in the SE localities of Leticia (LET), Puerto Nariño (PNA) and Tarapaca (TAR). Two different PCR-RFLP-ITS2 patterns were detected: one previously described for NW *An. triannulatus* s.l., present in both regions and a second pattern in which one band size differed from the expected value, only present in SE. For the *COI* analyses, a 1073 bp sequence for 198 specimens was analyzed. Haplotype diversities of 0.972+/-0.007 (NW specimens) and 0.991+/-0.003 (SE) and nucleotide diversities of 0.006+/-0.003 (NW) and 0.007+/-0.003 (SE) indicated high genetic variability. A F_{ST} value of 0.75 between NW and SE indicated high genetic differentiation. Statistical parsimony-based haplotype networks for each region and Fu's F_s test suggested population expansion or a selective sweep. Bayesian analysis with additional GenBank sequences of *An. triannulatus* s.s. from Meta department revealed three groups. Results of ELISA and confirmation by nested PCR showed five specimens naturally infected: two from BAG-NW with *P. vivax* VK247, two with *P. falciparum* from PLT-NW and PNA-SE, and one with *P. vivax* VK210 in TAR-SE. Preliminary results indicate that *An. triannulatus* s.l. may participate in malaria transmission and at least three lineages may be present in Colombia.

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USING PCR-DGGE TO INVESTIGATE THE BACTERIAL DIVERSITY IN FIELD-COLLECTED AND LAB-RAISED *AEDES ALBOPICTUS* AND THEIR EGGS

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Mosquitoes harbor a vast microbial community that plays an important role in digestion, reproduction and pathogen transmission. The observation that microbes interact with pathogens has lead to an interest in paratransgenic strategies where bacteria are manipulated to control disease transmission. Identifying possible candidates for these studies involves first characterizing the overall microbial diversity within mosquitoes. *Aedes albopictus* is an important emerging vector of both local and global mosquito-borne disease and has been implicated in recent outbreaks of Chikungunya in Italy and La Reunion Island. Accordingly, there is concern that if this virus were introduced into the United States, *Ae. albopictus* would spread it across the country. Thus, new and innovative control strategies are needed. PCR and denaturing gradient gel electrophoresis (DGGE) were used to profile and compare the bacterial communities between field-collected and lab-raised *Ae. albopictus* adults and their eggs. Eight different bacterial genera were identified from all adults, a subset of which was found in lab-raised adults. Adults had at least two, but never more than five genera present within them. Only one of these genera was present in eggs. The majority of bacteria identified were types readily sampled from the environment. There appears to be a reduction in species richness when a mosquito resides in a laboratory setting indicating that bacteria may be passively acquired while feeding as larvae or adults and are eventually lost. However, three genera were present in both field-collected and lab-raised adults, indicating a possible stable association of the bacteria within the mosquito. In this study, we were able to identify which bacteria infect and possibly associate with *Ae. albopictus*. The expanded knowledge resulting from this study is anticipated to aid in the development of future paratransgenic control strategies.

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THE IMPACT OF THE EXPANSION OF URBAN VEGETABLE FARMING ON MALARIA TRANSMISSION IN MAJOR CITIES OF BENIN

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Urban agricultural practices are expanding in several cities of the Republic of Benin. This study aims to assess the impact of such practices on transmission of the malaria parasite in major cities of Benin. A cross sectional entomological study was carried out from January to December 2009 in two vegetable farming sites in southern Benin (Houeyiho and Acron) and one in the northern area (Azèrèkè). The study was based on sampling of mosquitoes by Human Landing Catches (HLC) in households close to the vegetable farms and in others located far from the farms. During the year of study, 71,678 female mosquitoes were caught by HLC of which 25% (17,920/71,678) were *Anopheles* species. In the areas surveyed, the main malaria parasite, *Plasmodium falciparum* was transmitted in the south by *An. gambiae* s.s. Transmission was high during the two rainy seasons (April to July and October to November) but declined in the two dry seasons (December to March and August to September). In the north, transmission occurred from June to October during the rainy season and was vehicled by two members of the *An. gambiae* complex: *An. gambiae s.s.* (98%) and *An. arabiensis* (2%). At Houeyiho, Acron and Azèrèkè, the Entomological Inoculation Rates (EIRs) and the Human Biting Rates (HBRs) were significantly higher during the dry season in Households Close to Vegetable Farms (HCVF) than in those located far from the vegetable areas (HFVF) ($p < 0.05$.). However, there were no significant differences in HBRs or EIRs between HCFV and HFVF during the rainy seasons at these sites ($p > 0.05$). The knock-down resistance (*kdr*) mutation was the main resistance mechanism detected at high frequency (0.86 to 0.91) in *An. gambiae* s.l. at all sites. The *ace-1^R* mutation was also found but at a very low frequency (< 0.1). In conclusion, these findings showed that communities living close to vegetable farms are permanently exposed to malaria throughout the year, whereas the risk in those living far from such agricultural practices is limited and only critical during the rainy seasons. Measures must be taken by African governments to create awareness among farmers and ultimately decentralize farming activities from urban to rural areas where human-vector contact is limited.

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OUTDOOR HOST-SEEKING BEHAVIOR OF *ANOPHELES GAMBIAE* S.L. MOSQUITOES FOLLOWING INITIATION OF MALARIA VECTOR CONTROL ON BIOKO ISLAND, EQUATORIAL GUINEA

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Indoor-based anti-vector interventions remain the preferred means of reducing risk of malaria transmission in malaria endemic areas around the world. Despite demonstrated success in reducing human-mosquito interactions, these methods are effective solely against endophilic vectors. It may be that outdoor locations serve as an important venue of host-seeking by *Anopheles gambiae* sensu lato mosquitoes where indoor vector control measures are employed. We describe here the post-intervention host-seeking activity of anopheline mosquito vectors in the Punta Europa area of Bioko Island, Equatorial Guinea, where an IRS campaign has been underway since 2004 as part of the Bioko Island Malaria Control Program (BIMCP). We evaluated the venue and temporal characteristics of hosting-seeking by anopheline vectors in a hyperendemic setting using human landing collections conducted inside and outside homes in three villages during both the wet and dry seasons in 2007 and 2008. Additionally, human landing collections were performed as part of the BIMCP's vector monitoring activities throughout 2009. Collections were segregated hourly to provide a time distribution of host seeking behavior. Intense outdoor biting by *An. gambiae* senso stricto and *An. melas* vectors was observed throughout the night, including during the early evening and morning hours when human hosts are often outdoors. As reported previously, *An. gambiae s.s.* is the primary malaria vector in the Punta Europa region where it seeks hosts outdoors at least as much as it does indoors. Further, approximately 40% of *An. gambiae* s.l. are feeding at times when people are often outdoors, where they are not protected by IRS or ITNs. Repeated sampling over two consecutive dry-wet season cycles indicates that this result is independent of seasonality. In conclusion, *An. gambiae* s.l. mosquitoes currently seek hosts in outdoor venues as much as indoors in the Punta Europa region of Bioko Island. This contrasts with an earlier pre-intervention finding of exclusive endophagy of *An. gambiae* in this region. In light of this finding, we propose that the long term indoor application of insecticides has resulted in an adaptive shift to outdoor host seeking in *An. gambiae* s.s. on Bioko Island.

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BLOOD FEEDING PATTERNS OF *AEDES AEGYPTI* AND *AE. MEDIOVITTATUS* IN PUERTO RICO

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Aedes aegypti (L.) is often considered the principal mosquito vector of dengue viruses in Puerto Rico. However, there is another competent dengue vector on the islands that also has high rates of vertical transmission of virus in the laboratory. Aedes (Gymnometopa) mediovittatus, the Caribbean treehole mosquito, is native to the Greater Antilles and overlaps in distribution and aquatic habitats with *Ae. aegypti* in rural and urban areas with tall vegetation in Puerto Rico. It has been suggested that *Ae. mediovittatus* could act as a dengue virus reservoir or secondary vector, but it has never been found infected in nature. We studied the blood contents of both mosquito species to determine vector contact with humans and other vertebrates. The study was conducted in nine localities in southern Puerto Rico during 2010. Mosquitoes were collected outdoors in urbanized areas using BG-Sentinel traps baited with BG-lure. Three methods were used to identify the 609 blood meals that were obtained in this study: a multiplex PCR for humans, dogs, and cattle targeting cytochrome b, a PCR targeting the 16S rRNA, and a nested PCR targeting cytochrome b. Contrary to previous studies where *Ae. aegypti* fed almost exclusively on humans, this study showed that 78% took blood from humans, 19% fed from dogs, and the final 3% were represented by cats, chickens, and horses. Hosts for *Ae. mediovittatus* were 48% humans, 31% dogs, 7% cows, and 6% goats. The remaining 8% were represented by cats, horses, pigs, brown rats, chickens, and sheep. The results of this study indicate that *Ae. aegypti* does not feed exclusively upon humans in Puerto Rico, and shows that a large percentage of *Ae. mediovittatus* does feed upon humans.

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GENETIC DETERMINANTS OF INFECTION AND DISSEMINATION OF ENZOOTIC VENEZUELAN EQUINE ENCEPHALITIS VIRUS IN THE ENZOOTIC MOSQUITO VECTOR, *CULEX (MELANOCONION) TAENIOPUS*

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Venezuelan equine encephalitis (VEE) is a re-emerging mosquito-borne disease with the potential to cause fatal encephalitis in both humans and equids. VEE virus (VEEV) circulates in nature in two independent cycles: enzootic and epizootic. Epizootic, or epidemic, strains cause disease in equids and are vectored by a variety of mosquito species, including *Aedes taeniorhynchus*. Enzootic virus strains generally cause no disease in equids and circulate in a transmission cycle between primarily *Culex (Melanoconion) spp.* mosquitoes and rodent hosts. Recently, a higher number of human cases caused by enzootic strains have been detected in Mexico, Bolivia, Ecuador, and Peru emphasizing the importance of understanding the enzootic transmission cycle of VEEV. However, the majority of work examining the viral determinants of vector infection has been performed in the epizootic mosquito vector, *Aedes taeniorhynchus*. This work showed that envelope glycoprotein E2 is the primary determinant of vector competence for epizootic strains of VEEV. Given that the transmission dynamics of VEEV with enzootic and epizootic vectors are distinct, we hypothesized that the molecular determinants for infection of an enzootic mosquito vector, *Cx. (Melanoconion) taeniopus*, would not be the same for those of the epizootic vector. To examine this hypothesis we developed four reciprocal chimeric virus strains using representative epizootic and enzootic strains of VEEV, which differ dramatically in their ability to infect *Cx. taeniopus*, to examine the role of the nonstructural and structural proteins as well as the 3' un-translated regions (UTR) during infection and dissemination. All four chimeras showed statistically different infection rates when compared to both full-length parental strains indicating that both nonstructural and structural proteins contribute to enzootic vector competence. Interestingly, the IE/IAB chimera showed significantly higher infection when compared to each of the other chimeras (IAB/IE, IAB/IE/IAB, IE/IAB/IE), suggesting that the 3'UTR is important for specific infection of the enzootic vector.

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VECTOR COMPETENCE OF U.S. STRAINS OF THE ASIAN TIGER MOSQUITO, *AEDES ALBOPICTUS*, FOR CHIKUNGUNYA EPIDEMIC VIRUS (CHIKV 226OYP)

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In recent years the global spread of Chikungunya virus (CHIKV) by the invasive mosquito *Aedes albopictus* has been documented. In 2006, a new

genotype of the virus emerged which causes high rates of infection and dissemination in *Ae. albopictus*. In the past four years CHIKV has jumped from islands in the Indian Ocean to local transmission in temperate regions of Italy where *Ae. albopictus* was introduced in 1990. The potential now exists for the introduction of CHIKV to the United States given the wide and expanding distribution of *Ae. albopictus* in the Eastern United States. We evaluated the vector potential of three mosquito strains originating from Texas, New Jersey and Florida, and compared them with *Ae. albopictus* from Reunion Island. Infection and dissemination rates were high in all strains with highest rates in Florida and Reunion Island *Ae. albopictus*.

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CAN HORTON HEAR THE WHOS? SCALE IN VECTOR-BORNE DISEASE

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The epidemiology of vector-borne pathogens is affected by mechanisms and interactions at different scales, from individual level molecular processes to ecosystem interactions between species and their environment. This is of particular interest in the development of mathematical models to understand pathogen dynamics or develop intervention strategies. Choosing the scales and interactions included in models is critical for the conclusions drawn. We illustrate this using a key aspect of vector-borne disease, transmission of the pathogen between vectors and vertebrate hosts. A model of mosquito infection is expanded to illustrate the types of studies needed. Each mosquito has a number of virions needed for infection sampled from a gamma distribution and ingests a number of virions in its blood meal sampled from a separate gamma distribution. The two distributions are considered jointly in their effects on the resulting number of infectious mosquitoes. The parameters of the gamma distributions affected the number of infectious mosquitoes, with higher numbers occurring when the distributions were different. The assumptions about individual level characteristics (parameters of the gamma distributions) affected population level characteristics (number of infectious mosquitoes). Similar effects occur between other scales. Population interactions can affect community structure, while heterogeneity in community structure and population interactions with the environment can modify vector-borne disease transmission cycles. The interaction of communities of vectors, vertebrate hosts, and pathogens within the context of changing environmental conditions will influence individual life histories and population characteristics. Although complex, it is critical that interactions at different levels of scale are understood in order to fully integrate laboratory or small-scale field studies into an improved understanding of disease transmission at all scales, with the ultimate goal of improving risk prediction and reducing vector-borne disease.

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VERTICAL AND VENEREAL TRANSMISSION OF DENV VIRUS IN *AEDES AEGYPTI*

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Aedes aegypti is the most important vector of DENV to human hosts in nature. However, it has long been assumed that this species is not an important contributor to DEN viral persistence during low transmission periods. Vertical and venereal transmission are two mechanisms that could contribute to DENV persistance in nature. In recent years there have been several reports on vertical transmission potential in *Ae. aegypti* with confusing and sometimes contradictory results. In order to understand the role of vertical transmission in the natural history of DENV in Mexico, we investigated vertical transmission potential in the laboratory with a low generation, genetically diverse laboratory strain (GDLS) of *Ae. aegypti* from Chiapas, Mexico and DEN2J1499. We found a low but consistent rate of vertical transmission. In addition, we report, for the first time, venereal transmission of DENV from males to females during mating.

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SELECTION OF RESISTANT GUT FLORA IN CHILDREN DURING ACUTE RESPIRATORY INFECTION TREATMENT IN VIETNAM

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Viruses are the most common cause of acute respiratory infections (ARI) in children. However, the difficulty to clinically differentiate between viral and bacterial pathogens and the lack of rapid diagnostics causes doctors to prescribe antibiotics for all acute respiratory infections (ARI). Antibiotic use may select resistant gut flora which, in turn, can transfer resistance genes to other (pathogenic) bacteria within the gut and transmit from person to person. This study was undertaken to measure the selection of resistant gut flora in children reporting to an outpatient clinic with symptoms of ARI. ARI outpatients under 16 years of age were included and respiratory swabs were analyzed for the presence of pathogenic viruses and bacteria and in rectal swabs (day 0, 7 and 28) the fraction of resistant *Enterobacteriaceae* was assessed. History of medication and antibiotic use were recorded. In one year, 563 patients were enrolled. Five hundred sixty one (99,6%) patients received antibiotics, consisting mostly (94,1%) of beta-lactam drugs. Viral respiratory pathogens were identified in 488 cases (86.7%). After 1 week, a significant increase was found in the fraction of bacteria resistant to commonly used antibiotic classes as penicillins and cephalosporins, but also to rarely used classes as aminoglycosides and quinolones (likely caused by co-carriage of resistance genes on plasmids). Restoration was observed after 28 days. In conclusion, antibiotic use in children with ARI has a great impact on the bacteria in gut flora and causes temporary increased shedding of resistant bacteria. Restrictive use of antibiotic in children with ARI is recommended. The availability of rapid diagnostic tests that assess the presence of viral or bacterial pathogens in the respiratory tract may assist treating physicians in prudent decision making to help lower antibiotic prescription rates.

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PNEUMOCOCCAL ANTIGEN DIVERSITY IN HIV INFECTED ADULTS IN MALAWI

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Streptococcus pneumoniae is a major global health concern. Pneumococcal pneumonia accounts for a million deaths and more than 150 million episodes of pneumonia annually in children under five years. Populations most at risk of IPD are children under the age of 5 years, the elderly (> 65 years) and those with compromised immunity, such as the HIV infected. There are over 90 pneumococcal serotypes with distinct polysaccharide capsules, with only a few responsible for the majority of severe disease. Vaccines are an effective intervention, but more deaths could be averted by optimal use of vaccines with good coverage. Vaccines currently available target invasive serotypes associated with disease in Europe and the USA, and are not specific to and offer poor protection against African strains. This study aims to identify potential protein vaccine candidates from African strains. These protein antigens should have broad coverage able to elicit a robust complement-dependent bactericidal immune response.Comparative genomics allows the identification of different potential proteins candidates that are in such small quantities that they could not be purified and used as antigens by conventional vaccine development methods. Our dataset (currently n=84 draft genomes, 12 complete genomes) provides a comprehensive catalogue

of all potential vaccine candidates regardless of relative abundance or whether they are expressed under *in vivo* or *in vitro* conditions. We annotate the genomes and employ a clustering approach to identify similar proteins antigens. The data provides a clear picture of the species genetic diversity suggests differences in antigens between hosts.

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TUBERCULOSIS ACTIVE CASE DETECTION IN SENTINEL SITES ACROSS PAPUA NEW GUINEA

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In 2009 WHO reported a tuberculosis (TB) prevalence rate of 337 per 100,000 people and an incidence rate of 250 per 100,000 people for Papua New Guinea (PNG). This makes PNG to one of the high burden countries in the western Pacific region. However, the current estimate of the TB prevalence in PNG comes exclusively from non-systematically collected records from hospitals and health facilities, and therefore only represents the number of cases that were diagnosed and received treatment. This is most likely a gross underestimation of the TB prevalence in the country. This study aims to establish a community-based case prevalence of TB around several health centres across the country to obtain an estimate of the true burden of TB in PNG. Active TB case detection surveys are being conducted in four sentinel sites across PNG which were established in the frame of the Global Fund Round 8 Malaria Grant. In each site, people aged 15 years or above are screened for chronic cough from whom sputum samples are being collected. All suspected samples are screened for Mycobacteria by microscopy, tested for drug susceptibility to assess prevalence of drug resistance and subsequently genotyped to identify the major M. tuberculosis lineages and circulating bacterial strains. A complementary sample collection strategy through passive case detection is conducted in three urban hospitals. At the first two sites - Usino Bundi district (Madang) and Alotau district (Milne Bay)-, a total of 1736 households with 5038 members aged 15years or above were screened. A total of 164 people with chronic cough were identified and their sputum analysed. Data on community prevalence, age and sex distribution of pulmonary tuberculosis infection as well as preliminary data on the prevalence of drug resistance and on prevalence of different Mycobacterium tuberculosis genotypes in the study areas will be presented.

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SEROPREVALENCE OF PERTUSSIS IN CHILDREN OF NORTHERN SENEGAL

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Pertussis, also known as whooping cough, is a vaccine-preventable respiratory disease caused by *Bordetella pertussis* infection, against which Senegalese children are immunized with the Diphteria-Tetanus-whole Pertussis vaccine (*DTwP*). Seroepidemiology of pertussis has been widely described in industrialized countries, but rare are the studies referring to it in developing countries. We conducted a longitudinal survey in Northern Senegal to investigate the epidemiology of *B. pertussis* by evaluating the IgG antibody (Ab) titers to two of its antigens (Ag): filamentous hemagglutinin (FHA) and pertussis toxin (PT). A cohort of 410 children aged 1 to 10 from 5 villages in the Senegal River Valley was followed-up for 18 months. During that period, 5 visits have been made to the villages to assess the immunological status of the children. Both FHA and PT-specific IgG responses were significantly different according to age. This is in accordance with observations from Western countries in which age is known to be an important factor in the epidemiology of pertussis. Until the age of 5, Ab response to FHA was low, and got higher in the older group. Assessment of anti-PT IgG response suggested evidence of recent exposures to the pathogen, especially in the older group. Surprisingly, in one of the five villages the average Ab response to both FHA and PT was very low at all tested ages during the first 6 months of the study. However, at the third visit, anti-PT Abs peaked to very high levels, to slightly decline at the end of the survey. This indicates an epidemic of *B.pertussis* in that particular village, whereas in the other four villages an endemic profile could be observed. Thus, pertussis continues to be endemic in Northern Senegal despite the introduction of immunization. This serological survey gives information that could not be captured by disease notification data, mainly because the disease is under-notified in older children and adults in which a pertussis infection can be easily missed due to the atypical forms of the disease in those age categories. This under-diagnosis of pertussis ensures that the disease remains endemic, posing a threat to vulnerable infants at great risk of morbidity and mortality from whooping cough. Therefore, a more complete understanding of the epidemiology of pertussis could provide information to adapt health programs in order to target more adequate age groups for immunization.

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DIAGNOSTIC AND TREATMENT DELAY OF PULMONARY TB IN SELECTED RURAL AND URBAN AREA OF BANGLADESH

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Delay in diagnosis and initiation of treatment of pulmonary TB results in severe disease and increased burden of the disease in Bangladesh where disease prevalence is high. This study explored the underlying factors that influence delay in diagnosis and treatment of pulmonary TB among adults. Newly diagnosed sputum positive TB patients aged 15 years and above attending selected urban and rural TB clinics were interviewed. First two patients attending TB centers were enrolled each day during May to September 2010. Total delay comprises of patient delay (from first symptom to first visit to qualified doctors) and health care system delay (from first visit to qualified doctor to TB diagnosis and treatment initiation). A total of 278 patients were enrolled, 151 from urban Dhaka and rest from rural Mirzapur sub-district. The median total delay was 62 days (Inter quartile range 37, 96 days). Patient delay was significantly more in rural area compared to urban area and health care system delay was longer in urban area compared to rural area ($p< 0.001$). Higher degree of stigma (OR 2.0; 95% CI 1.2-3.4; $p=0.008$) and older age group (OR 3.3; 95% CI 1.7-6.4, $p<0.001$) were associated with longer delay in TB diagnosis and treatment. There is an urgent need for improving comprehensive service delivery to reduce the total delay in diagnosis and treatment of pulmonary TB in Bangladesh, particularly patient delay in rural areas and health care system delay in urban areas. Stigma still prevents patients from seeking care and should be alleviated by increased awareness building. This will also help in motivating the elderly to seek care early on during the disease.

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IMPROVING DIAGNOSIS AND SURVEILLANCE OF CHILDHOOD TB IN KENYA

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Globally paediatric TB remains a common but neglected cause of childhood illness and death. Both surveillance and individual case management are severely compromised by difficulties in diagnosis of TB in

children, particularly in low resource settings where diagnostic facilities are lacking and the burden of disease is often highest. Two urgent research priorities for childhood tuberculosis are therefore (1) to collect robust regional data on the true burden of paediatric TB; and (2) to improve paediatric TB diagnosis, and in particular to develop a rapid, reliable and affordable diagnostic test for use at the point of care in resource poor settings. Kenya is among the 22 high burden TB countries globally and has one of the highest estimated incidence rates of TB among children; however good quality data on the true burden of TB are lacking. We utilized a continuous demographic surveillance survey combined with state of the art laboratory TB diagnostics, intensified case finding and careful clinical follow up to estimate the true incidence of childhood TB in an area of rural Kenya; and to investigate the performance of existing clinical and laboratory tools for the diagnosis of childhood TB in this setting. We present our preliminary findings from 2 years surveillance during which over 1500 children were investigated for suspected TB. There was heterogeneity in the performance of existing clinical diagnostic algorithms for childhood TB, but all were limited by either poor sensitivity or poor specificity. Smear microscopy of induced sputum specimens was highly insensitive but both automated liquid mycobacterial culture and Microscopic Observed Drug Susceptibility (MODS) culture significantly increased diagnostic yield. The estimated crude incidence of childhood TB is lower than previous published estimates extrapolated from national notification data but is likely to be an underestimate. We explore methods to estimate the degree of case under-ascertainment and adjust incidence estimates accordingly. Utilizing well characterised cohorts of children with and without active TB our ongoing work investigates the potential role of serum biomarkers for paediatric TB diagnosis in this population, using proteomics and RNA expression profiling. [Note: Recruitment to the study will continue until mid 2011. The most up to date data will be presented at the meeting; exact figures are therefore omitted from the abstract.]

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INFECTIOUS DISEASE MORTALITY ASSOCIATED WITH REGIONAL MOVEMENT OF PACIFIC ISLANDERS IN 19TH-20TH CENTURIES

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Many small Pacific Islands experienced loss of up to 90% of pre-contact populations within several decades of arrival of Western explorers, traders, and/or military in the late 18th to early 20th centuries. Although it is known that epidemic infectious diseases contributed to this extreme mortality, why it occurred on these isolated islands remains unknown. Since enumeration often did not begin until after initial lethal epidemics, we sought out special groups with good mortality records in order to better understand first-contact population losses. Specifically the historical records of indentured labourers largely from Melanesia to sugar plantations either in Queensland or Fiji were examined. Annual mortality of Pacific Islanders in Queensland and Fiji was up to 10% causing continued close attention to mortality of such labourers. Mortality largely occurred soon after leaving their native island falling from 14% in the first year to 5% in the second year and <3% in the third year. This was considerably higher than Indian labourers working in the same Fijian sugar plantations whose mortality decreased from 4% in the first year to <2% in the fifth year. Most deaths were due to pneumonia/influenza and dysentery. Pacific islander mean annual all-cause mortality fell over the decades that the system of indentured labour existed being 8% in 1879-1887, 5% in 1888-1892 and 3.5% in 1893-1906. These figures were remarkably consistent between Queensland and Fiji whereas Indian labourers at the same time experienced less than half these mortality rates (range 1.8-3.6%) on Fijian sugar plantations. These data are interpreted to indicate that isolated Pacific island populations were intrinsically vulnerable to lethal infectious diseases particularly pneumonia and that this mortality moderated for both entire populations and individuals over time. Since this occurred in the pre-antibiotic era, it seems likely that genetic susceptibility explains much of the lethality seen and that this vulnerability may be due to the successive population bottle-necks experienced in migrating Polynesian and Melanesian populations.

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SOCIAL AND DEMOGRAPHIC PROFILE OF PATIENTS HOSPITALIZED IN 2010 AT A HOSPITAL OF TUBERCULOSIS IN BRAZIL

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Tuberculosis remains a public health emergency in many countries, including Brazil. Although currently used as an alternative to hospitalization of patients is still important in controlling the disease. Nestor Goulart Reis Hospital with 100 beds, is one of two in the State of Sao Paulo, which is the richest state of the Federation. It was performed demographic and social caracterization of all patients admitted to this hospital in 2010 from the records. From a total of 114 admissions, 82.5% men and 17.5% women. Among the patients, 9.7% were homeless, all of them were Male. Young adults were predominant (20-39 years) from 53.5% of the total, followed by other adults (40 to 59 years), with 39,5%. Regarding marital status, the vast majority of patients lived alone, with 75.4% single. The education level of patients was low compared to the general population, with 9.7% of illiterates, 41.3% with 4 years of study and 34.2% with up to 8 years of study. Regarding to diagnosis, 93.9% were pulmonary tuberculosis, 5.3% of multidrug-resistant pulmonary tuberculosis and one case of extra-pulmonary tuberculosis. Regarding the co-prevalence of other diseases, 71.0% suffered from alcoholism, drug abuse 15.8%, 9.0% suffered from AIDS, hepatitis C 12.3%, 7.9% for syphilis, 5 3% of blastomycosis, 4.4% of psychiatric illness and 4.4% of various nutritional disorders. Regarding to the hospital releasing 69.4% were due to cure or continuing treatment at home, 18.4% abandoned the treatment and 7.9% died. Regarding to employment status, 70.2% of patients were unemployed and 15.8% were out of their jobs due to the hospitalization. These numbers show the great importance of tuberculosis hospitals, once the hospitalized patients a have a specific pattern, which are: predominance of people living in situations that difficult the outpatient treatment of the disease such as alcoholism, drug addiction, lack of family ties, poor education and unemployment.

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PREVALENCE OF SKIN TUBERCULIN TEST (PPD) POSITIVE AMONG WORKERS OF A TUBERCULOSIS HOSPITAL IN BRAZIL

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The occurrence of tuberculosis among professionals working in hospitals is higher than in the general population, including the risk of outbreaks in these occupational groups. For this reason it is important to estimate the prevalence of infection by M. tuberculosis, among the professionals who are in permanent contact with tuberculosis patients. An effective way of estimating this prevalence is by skin tuberculin test (PPD), which tends to increase their levels of positivity to the extent that it increases the contact time with patients. In 2005 were invited to undergo this test all professionals working in the Nestor Goulart Reis Hospital, in São Paulo, Brazil. This is a public hospital, since 1958 specialized in the treatment of tuberculosis. From the total of 250 professionals, 137 agreed to submit proof of PPD, including from workers with closer contact with patients, nurses, dentists and other psychologists, to others with little or no contact with patients, the administrative area of the institution. The test was conducted to PPD Rt 23, the reading occurring 72 hours after injection, by palpation. In duration greater than 5 mm were considered positive, those reactions with up to 4 mm were considered negative and re-tested after several weeks. Results 5-9 mm were considered weak reactors, from 10 mm were considered strong reactors. Among the 137 employees who

agreed to conduct the test, 129 took the reading test. Of these 69.0% were reactive to PPD, with 61.24% and 7.76% were strong reactors weak reactors. The average age of the employees tested was 46 years and mean duration of hospital services to the age of 13 years. Regardless of the industry they worked, the prevalence of positive PPD was high, whether or not directly related to patient care. The results justify the adoption of strict biosecurity measures in hospitals specialized in the treatment of tuberculosis at high risk of infection exist in these environments.

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PRIMARY *PLASMODIUM YOELLI* NL MALARIA INFECTION DOES NOT REDUCE NEW TB VACCINE EFFICACY IN A MURINE MODEL OF TUBERCULOSIS

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In our laboratory, animal models for studying TB and malaria have been established, and are being used to test the safety, immunogenicity, and effectiveness of new vaccine candidates. Based on this expertise, we have designed studies to determine i) whether BCG and new candidate TB vaccines elicit effective protective immune responses following *Mycobacterium tuberculosis* Erdman aerosol challenge of mice which have been previously infected with *Plasmodium yoelli* NL malaria parasites, and ii) the immunomodulatory effects that Py NL infections have in BCG vaccinated mice. When BCG vaccinated mice were challenged with MTB by the aerosol route at the peak of the Py NL infections, no impact was seen on the protective efficacy of BCG. In addition to BCG, new Mtb vaccine candidates and immunizations strategies were also tested. Mice were vaccinated with: i) a recombinant E6-85 (ESAT6 and Ag85B) fusion protein in DDA/MPL adjuvant, ii) a E6-85 + DDA/MPL prime, MVA-5MTB (ESAT6/Ag85A/Ag85B/HSP60/Mtb39) and Il-15 boost combination, and iii) a ΔSecA/LysA Mtb deleted mutant. Again, malaria co-infections do not impair lung protection conferred by new Mtb vaccine candidates. Since it is known that *Plasmodium* parasites can inhibit some immune functions, the effect that Py NL malaria infection has on the immunity induced by the BCG vaccinations was assessed using flow cytometry. Results showed that specific BCG vaccine-induced pulmonary cell-mediated immune responses were suppressed by active Py NL infections, but after Py NL parasite clearance, the cellular frequencies and the median fluorescent intensity values of CD4 and CD8 T cell subsets of the BCG vaccinated and the BCG vaccinated-malaria infected mice were not significantly different. In conclusion, CFU results showed that malaria co-infections do not impair lung protection conferred by BCG or new Mtb vaccine candidates. Moreover flow cytometry results suggest that the inhibition of BCG-induced T cell function by a primary *P. yoelii* infection is short-lived and this malaria-induced suppressive activity wanes after parasite clearance.

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HOUSEHOLD FACTORS ASSOCIATED WITH INDOOR AIR POLLUTION IN A LOW-INCOME URBAN AREA IN BANGLADESH

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Indoor air pollution is a significant contributor to respiratory infections in resource-poor countries. In low-income settings where biofuel use is uncommon, there is little information about associations between household structural factors, such as ventilation and building materials, and fine particulate matter ($PM_{2.5}$) concentrations. In the control population of a case-control study on risk factors for pneumonia in a low-income community in Dhaka, Bangladesh, we sought to identify household factors associated with high levels of indoor air pollution. We interviewed primary caregivers of young children about fuel use and assessed ventilation, roof and wall material, and location of stoves. We measured $PM_{2.5,}$ in the living space for 24 continuous hours. We defined high $PM_{2.5}$ as $PM_{2.5}$ exceeding 250 μg/m^3 for 40 minutes or more, with the cutpoint of 40 minutes chosen based on the median duration of exposure to ≥250 μg/m^3, a concentration of PM that is 10 times the WHO guideline for 24-hour indoor exposure. We used logistic regression to estimate associations between household factors and high $PM_{2.5}$ after adjusting for socioeconomic status. The mean of the 24-hour geometric mean $PM_{2.5}$ in living spaces was 98μg/m^3 in the 663 households. Biofuel, such as wood instead of liquefied petroleum gas, was used overall by 9%, and was significantly more commonly used in the high $PM_{2.5}$ group (17%) than in the low $PM_{2.5}$ group (1%) [OR_{adj}=13.2 95% CI=3.0, 58.3]. Distance from the stove to the living space was inversely associated with high $PM_{2.5}$ (OR_{adj} 0.97 per step, 95% CI=0.94, 0.99). Number of walls around the kitchen was inversely associated with high $PM_{2.5}$ in the living space (OR_{adj} 0 walls v. 4 walls = 17.4 95% CI 2.1, 143.9). Cross-ventilation, number of walls with windows or doors, and wall and roof materials of the living space were not associated with $PM_{2.5}$ after adjustment for SES. Biofuel use, while associated with indoor air pollution, is relatively uncommon in the study community. Our findings indicate that cooking-related emissions contribute to indoor air pollution even in households using improved fuels. We recommend an exploration of whether these structural factors are modifiable in ways that are feasible and acceptable. To better design interventions to reduce respiratory infections in low-resource settings, we must understand more fully the causes of indoor air pollution in polluted urban areas where biofuel use is uncommon.

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MOLECULAR INSIGHTS FOR *GIARDIA, CRYPTOSPORIDIUM* AND SOIL TRANSMITTED HELMINTHS FROM A FACILITY-BASED SURVEILLANCE SYSTEM IN GUATEMALA

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Parasitic infections are common causes of gastrointestinal infections worldwide. Microscopy methods can properly identify helminths and some protozoa, however cannot provide further information. In this study we further characterized microscopy-positive fecal specimens collected from 645 patients with diarrhea, from January to March 2006, in a facility-based surveillance study in Guatemala. Samples positive for *Giardia duodenalis* and *Cryptosporidium spp*, were genotyped to gain knowledge on their transmission dynamics. Samples with the soil transmitted helminths (STH) *Ascaris lumbricoides* and *Trichuris trichiura,* were sequenced at the β-tubulin locus to investigate resistance against benzimidazolic (BZ) drugs. Specimens were microscopically analyzed for ova and parasites, and acid-fast stain for *Cryptosporidium* spp. Microscopy-positive samples were PCR-amplified at informative loci: triose phosphage isomerase of *Giardia* (TPI), SSU rRNA and GP-60 of *Cryptosporidium,* and β-tubulin of *A. lumbricoides* and *T. trichiura*, and the resulting amplicons were sequence-analyzed. Thirty-five specimens (5.4%) had *Giardia*, five (0.8%) *Cryptosporidium,* 37 (5.7%) *A. lumbricoides*, and 13 (2%) *T. trichiura*. Twenty samples were successfully genotyped at the TPI locus. Assemblages A and B in seven (35%) and 12 (60%) of specimens were identified respectively, while one sample had both assemblages. Four samples with *Cryptosporidium* were successfully genotyped: *C. hominis* (n=2) and *C. parvum* (n=2). The characterization of *A. lumbricoides* and *T. trichiura* was accomplished from 32 and nine samples respectively. All STH samples had the homozygous codon TTC, associated with sensitivity to BZ. The molecular data of *Giardia*

and *Cryptosporidium* showed parasite diversity, as well as evidence of anthroponotic and zoonotic transmission. These findings highlight the importance of molecular tools in public health activities.

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ACANTHAMOEBA KERATITIS OUTBREAK IN CHICAGO, ILLINOIS IS ASSOCIATED WITH THE PRESENCE OF THE PATHOGENIC BACTERIA *LEGIONELLA PNEUMOPHILA*

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Acanthamoeba is a protist which causes a rare sight threatening eye infection, Acanthamoeba keratitis (AK). A dramatic increase of AK in conjunction with discovery that *Acanthamoeba* can harbor pathogenic bacteria as endosymbionts has heightened public health concerns. *Acanthamoeba* may act as a "trojan horse" of many different types of bacteria including Legionella, the causative agent of Legionnaires Disease. In Acanthamoeba, these bacteria multiply and are released into the environment, facilitating transmission to humans. Also, *Acanthamoeba* can survive harsh conditions including most drug, allowing the bacteria to survive within *Acanthamoeba* when it otherwise would have been destroyed. Since 2003, the incidence of Acanthamoeba keratitis has increased dramatically in many metropolitan locations including Chicago, Illinois. These increases have been hypothesized to be a result of recent EPA mandated water treatment changes that has increased the biofilm in the water system and the prevalence of *Acanthamoeba*, which feeds on biofilm. Previous data has confirmed the keratitis-causing *Acanthamoeba* are not a novel or more pathogenic species. We hypothesized that keratitis-causing *Acanthamoeba* in Chicago patients may be associated with *Legionella*, which increased its virulence and therefore its capacity to cause disease. 47 clinical samples of *Acanthamoeba* from keratitis patients from Chicago from 2005-to present were tested for the presence of *Legionella* using *Legionella* specific primers to amplify an internal portion of the 16S ribosomal RNA gene via PCR. Positive samples were confirmed by DNA sequencing. Of 47 clinical samples, 28 tested positive for *Legionella*. Sequence analysis confirmed the presence of Legionella pneumophila in all bacteria-harboring Acanthamoeba. In situ hybridization confirmed the presence of these bacteria intracellularly in the Acanthamoeba. This data shows a surprisingly high amount of bacteria associated with disease causing Acanthamoeba which suggests a roll for pathogenic bacteria in the virulence of *Acanthamoeba*.

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ASSESSMENT OF A NEW PARASITOLOGY SCREENING DIAGNOSTIC ELISA FOR THE DETECTION OF ANTIGENS OF *GIARDIA* SPP., *CRYPTOSPORIDIUM* SPP. AND *ENTAMOEBA HISTOLYTICA* IN FECAL SPECIMENS

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Giardia spp., *Cryptosporidium* spp., and *Entamoeba histolytica* are among the most common protozoan sources of parasite-associated diarrheal disease worldwide. A lack of rapid and cost-effective diagnostic tools is a major challenge to the surveillance of disease caused by these three pathogens. The development of the *TRI-COMBO PARASITE SCREEN* ELISA by TechLab, Inc. that can simultaneously detect antigen for these parasites in clinical stool samples represents a significant advantage in screening for these pathogens. Evaluation of the *TRI-COMBO* test is currently underway at three tropical medicine reference centers, the National Institutes of Infectious Diseases(NIID) in Tokyo, Japan, the International Center for Diarrheal Disease Research, Bangladesh(ICDDR,B), in Dhaka, Bangladesh, and the Bernhard Nocht Institute for Tropical Medicine in Hamburg, Germany. To date, 400 clinical samples have been subjected to analysis by the *TRI-COMBO* test and compared to the *GIARDIA II*, *CRYPTOSPORIDIUM II*, and *E. HISTOLYTICA II* individual stool ELISA tests from TechLab. Out of this panel of samples, the *TRI-COMBO* test detected 161 samples positive for *Giardia* spp., *Cryptosporidium* spp., and/or *E. histolytica*. The *GIARDIA II*, *CRYPTOSPORIDIUM II*, and *E. HISTOLYTICA II* individual stool ELISA tests detected 81 samples positive for *Giardia* spp., 35 samples positive for *Cryptosporidium* spp., and 47 samples positive for *E. histolytica*. 10 samples were positive for more than one parasite, as confirmed by detection with the individual ELISA format tests and 10 samples were found to be positive on the *TRI-COMBO* test but negative on the individual stool ELISA tests. 2 samples were found to be negative on the *TRI-COMBO* test while positive on the individual stool ELISA tests. 237 samples were confirmed negative on all tests. In conclusion, the *TRI-COMBO* test displayed 98.7% sensitivity and 95.95% specificity during screening of a large number of clinical samples for the presence of *Giardia* spp., *Cryptosporidium* spp., and *E. histolytica*.

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EVALUATION OF A NEW RAPID DIAGNOSTIC TEST FOR THE DETETION OF *GIARDIA* SPP. AND *CRYPTOSPORIDIUM* SPP. IN HUMAN FECAL SPECIMENS

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Giardia spp. and *Cryptosporidium* spp. are pathogenic protozoan parasites able to colonize the human intestine and are among the leading causes of traveler's diarrhea. Here, we report the clinical evaluation of the *GIARDIA/CRYPTOSPORIDIUM QUIK CHEK*, a rapid point of care assay capable of simultaneously diagnosing infection of these organisms through the identification of antigen in human fecal specimens. The test involves a membrane-based device with immobilized capture antibodies and a soluble peroxidase-conjugated antibody that is combined with a diluted specimen. Only a simple dilution is necessary, with no filtering or centrifugation required. The diluted sample is then added to the membrane device, with time to result being less than 30 minutes. The assay result is a visible line for a positive result and the absence of a line for a negative result. No equipment is required for the assay or interpretation. The sample panel included 511 samples tested at both LSG & Associates and TechLab, Inc. Specimens tested at LSG & Associates were part of a panel of preserved fecal specimens obtained following routine patient testing. All samples tested were preserved in either 10% formalin or SAF. Specimens tested at TechLab, Inc. were originally submitted to a local clinical diagnostic laboratory for routine microbiology testing. These samples were fresh (undiluted) or preserved in either 10% formalin or sodium acetate formalin (SAF). All rapid test results were compared to microscopy using a direct immunofluorescent detection procedure (MERIFLUOR *Cryptosporidium/Giardia*). The evaluation included 431 preserved (215 10% formalin and 216 SAF) and 80 unpreserved fecal specimens. The *Giardia* line compared to IFA had sensitivity = 98.6%, specificity = 100%, and correlation = 99.6%. The *Cryptosporidium* line compared to IFA had sensitivity = 100%, specificity = 99.7%, and correlation = 99.8%. The simple format and rapid detection ability of this test makes it ideal for a variety of uses: small or large-scale screenings, field diagnostics, and use in developing countries.

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TRNA GENE STUDIES FOR GENOTYPING OF *ENTAMOEBA HISTOLYTICA* IN STOOL SAMPLES

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Entamoeba histolytica tRNA-linked STR (short tandem region) can be useful to establish a correlation between genotype of the parasite and the outcome of infection. A total of 662 stool samples from asymptomatic subjects (n:488) and suffering from diarrhea (n:174) in Diyarbakir, Turkey, were examined for the presence of *E. histolytica* using microscopy, stool antigen ELISA, conventional PCR, and real-time PCR from July 2008 through June 2010. *E. histolytica* was detected in 3.3% (22/662) of the stool samples by real-time PCR. Clinically, diarrhea was mostly prevalent in patients with positive testing by *E. histolytica* real-time PCR assay (20 associated with diarrhea/dysentery, but 2 associated with no symptoms). Parasite load can be correlated with clinical outcome in *E. histolytica* infected patients, since a parasite load of 10^3 copy/µl and than higher was detected mostly in patients with diarrhea. We investigated the tRNA - linked STR gene polymorphism in clinical isolates of *E. histolytica*. In the present study, we identified three different genotypes among 22 isolates when two loci (SD and SQ) were used. PCR amplification was not observed at the other loci (A-L, N-K, D-A, and R-R) in genotyping. We observed a limited degree of STR polymorphism among *E. histolytica* strains obtained from stool samples, even in the strains isolated from a restricted area. It was found that SD and SQ loci seem to be suitable in the study of tRNA-based genotyping of *E. histolytica.*

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DIAGNOSIS OF *GIARDIA* AND *CRYPTOSPORIDIUM* ENTERIC INFECTIONS WITH A NEW POINT-OF-CARE RAPID ASSAY

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Giardia spp. and *Cryptosporidium* spp. are pathogenic protozoan parasites able to colonize the human intestine and are among the leading causes of traveler's diarrhea. Infection can result in chronic debilitating diarrhea, nutrient malabsorption, and human-to-human transmission, making diagnosis a high priority for fecal testing laboratories. Here, we report the clinical evaluation of the *GIARDIA/CRYPTOSPORIDIUM QUIK CHEK*, a rapid membrane-based assay capable of detecting *Giardia* cyst antigen and *Cryptosporidium* oocyst antigen in human fecal specimens. Specimens tested were obtained by the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) from a cohort of children in an area where *Giardia* and *Cryptosporidium* infections are prevalent. The test utilizes immobilized capture antibodies and a soluble peroxidase-conjugated antibody that is combined with a diluted specimen. Only a simple dilution is necessary, with no filtering or centrifugation required. The diluted sample is then added to the membrane device, with time to result being less than 30 minutes. A visible line is required for a positive result and the absence of a line for a negative result. No equipment is required for the assay or interpretation. Results from the rapid test were compared to two FDA-cleared ELISA tests specific for *Giardia* and *Cryptosporidium*. The evaluation utilized 129 fresh and 180 frozen specimens (including 89 *Giardia* positives and 44 *Cryptosporidium* positives). The *Giardia* line compared to ELISA had 100% positive agreement, 100% negative agreement, 100% overall agreement. The *Cryptosporidium* line compared to ELISA had 100% positive agreement, 100% negative agreement, 100% overall agreement. The ease of use and rapid detection ability of this test makes it desirable to use in a variety of settings: small or large-scale screenings, field diagnostics, and use in developing countries. The data from this clinical evaluation indicate that the assay is a reliable method for the identification of *Giardia* and *Cryptosporidium* in unpreserved human fecal specimens.

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EVALUATION OF A POINT-OF-CARE SEROLOGY ASSAY SPECIFIC FOR THE DETECTION OF *ENTAMOEBA HISTOLYTICA*

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Entameoba histolytica is a protozoan parasite that infects over 50 million people annually resulting in approximately 100,000 deaths. Ingested cysts cause diarrhea and colitis; infection may lead to extra-intestinal symptoms such as brain and liver abscesses (ALA - amebic liver abscess). Serological tests for *E. histolytica* can be used as a marker of current and past infection. Here, we evaluate an *E. histolytica* rapid test and ELISA; both capable of detecting anti-*E. histolytica* adherence lectin antibodies in human serum. The membrane-based rapid test utilizes immobilized rLecA, a recombinant fragment of adherence lectin, on the membrane and a soluble peroxidase-conjugated anti-human IgG detection antibody. Similarly, the ELISA uses immobilized rLecA and the same detection antibody. Specimens were collected and screened at the International Centre for Diarrheal Disease Research (Dhaka, Bangladesh) and the Bernhard Nocht Institute (Hamburg, Germany). Results were compared to physician assessment of ALA, RT-PCR, and/or two laboratory assays previously described in the reviewed literature as specific for identifying human anti-*E. histolytica* antibodies in serum ("laboratory assay"). The rapid test was evaluated using 186 serum specimens resulting in 96.9% sensitivity and 100% specificity. Of the 186 specimens, 88 indicated negative serum titers with the laboratory assay, while 43 specimens indicated positive serum titers. ALA- and PCR-confirmed positive patients comprised the remaining 55 specimens. The ELISA was evaluated using 140 specimens resulting in 95.7% sensitivity and 87.1% specificity. Of the 140 specimens, 70 indicated negative titers with the laboratory assay, while 30 specimens indicated positive serum titers. ALA- and PCR-confirmed patients comprised the remaining 40 specimens. Results indicate that the *E. histolytica* serum rapid test and ELISA correlate with established clinical diagnosis. The simple format of the rapid test and large scale screening capability of the ELISA make these assays ideal for field diagnostics and use in developing countries.

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BURDEN AND FACTORS ASSOCIATED WITH *GIARDIA* INFECTION IN INFANTS OF SOUTH INDIA

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Giardia intestinalis is a common gastrointestinal protozoan worldwide. Infection varies from asymptomatic episodes to acute diarrhea, and it may persist as chronic diarrhea leading to malnutrition and growth failure in early infancy. In this study, we estimated the incidence rates and investigated risk factors for *Giardia* infection and their influence on growth at one year of age in a birth cohort of children (N=340) from an urban slum community in Vellore, South India. Intensive bi-weekly field surveillance visits were made for collection of morbidity data, diarrheal and surveillance samples and monthly anthropometric measurements. There were 107 episodes of giardial infection in 60 children. Twelve of these children had *Giardia* associated diarrhea, 33 had only asymptomatic infection and 15 children had both. Children were more likely to have

asymptomatic infection than symptomatic infection (Ratio=2:1). The median age (IQR) for first *Giardia* diarrhea was 10 (7-11) months. The median age (IQR) for the first *Giardia* asymptomatic infection was 8 (7-10). Overall incidence of *Giardia* infection was 33.02 episodes /100 child years (95% CI=27.32-39.91), with symptomatic infections at11.42 episodes/100 child years (8.27-15.76), and asymptomatic *Giardia* with a rate of 21.60 episodes/100 child years (17.09-27.30). In Poisson regression model the factors significantly associated with *Giardia* infection were presence of one or more siblings (RR=2.82, 95% CI 1.78-4.84), lower socio economic status (1.51, 0.98-2.33) and wasting (weight-for-height Z score <2) (2.53, 1.71-3.76). There was no association between stunting (RR=0.86, 95% CI 0.54-1.39) and giardiasis. The overall incidence suggests a high burden of giardiasis during infancy in this community. There is also evidence of association between giardiasis and acute malnutrition, although there was no apparent effect on chronic malnutrition.

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EVALUATION OF A DIAGNOSTIC SCREENING ELISA FOR THE DETECTION OF *GIARDIA* SPP., *CRYPTOSPORIDIUM* SPP. AND *ENTAMOEBA HISTOLYTICA* IN HUMAN FECAL SAMPLES

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The *TRI-COMBO PARASITE SCREEN* test is a diagnostic ELISA for the detection of *Giardia* spp, *Cryptosporidium* spp. and *Entamoeba histolytica* in human fecal specimens to aid in diagnosis of giardiasis, cryptosporidiosis and/or amebiasis. Identification of these parasites, the three most common enteric protozoan parasites worldwide, often involves microscopy which is labor-intensive, time-consuming and requires advanced training. Here we report results of a clinical evaluation of the *TRI-COMBO PARASITE SCREEN* test, a qualitative ELISA which offers a simple, highly sensitive and specific method of screening fecal specimens to identify those specimens positive for one or more of these parasites, eliminating the need for expensive microscopy methods on the majority of specimens. Positive results are indicated by the presence of a yellow color in the wells that can be interpreted visually or analyzed spectrophotometrically. A positive result indicates the presence of cysts or antigen from *Giardia* spp., *Cryptosporidium* spp., and/or *E. histolytica*. Clinical evaluations are currently underway for this test in order to seek FDA clearance. Fecal specimens are being tested on the *TRI-COMBO* test and individual commercial ELISAs specific for *Giardia* spp., *Cryptosporidium* spp. and *E. histolytica*. 75 specimens have been tested thus far in this study (35 positive for one or more parasites and 40 negative for all three parasites), and the sensitivity and specificity to date are 100%. The study goal is to test 250 specimens. Previous studies with the TRI-COMBO test have been conducted in our laboratory and in a birth cohort study at the ICDDR in Dhaka, Bangladesh. Results with 950 specimens demonstrated assay sensitivity and specificity in excess of 98% compared to individual ELISAs. The *TRI-COMBO* test can be used as a cost-effective screening assay to eliminate negative specimens and identify *Giardia*-positive, *Cryptosporidium*-positive and *E. histolytica*-positive specimens requiring further parasitological analysis.

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DETECTION AND GENOTYPING OF *CRYPTOSPORIDIUM* AND *GIARDIA* SPECIES IN PUBLIC PLACES IN SOUTHEASTERN OHIO

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Cryptosporidiosis and giardiasis are one of the most common causes of protozoal diarrhoea worldwide, and cause of significant morbidity and mortality in both the developing and developed world. The aim of this study is to investigate contamination of public places by *Cryptosporidium* and *Giardia* species. A total of 170 soil samples were collected from four school playgrounds and two public parks in Zanesville OH in 2008 and 2009. These samples were screened for *Cryptosporidium* and *Giardia* species cysts using a modified direct immunofluorescence assay. *Cryptosporidium* species oocysts were seen in 15 samples (9%) while 7 samples (4%) were positive for *Giardia* species cysts. Several molecular markers are being used for genotyping of these samples. Our results underlie the significance of public places in the transmission of these emerging protozoan infections and will add to our understanding of the contributions of humans and other reservoirs of infection to the epidemiology and risk assessment of the transmission of these diseases.

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MAPPING THE 2010 CHOLERA EPIDEMIC IN THE FAR NORTH REGION OF CAMEROON

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Cholera is caused by consuming water and food contaminated by *Vibrio cholera* and often spreads as a result of poor sanitation and hygiene. Cholera has long been, and continues to be, a world health issue. In 2010, a major cholera outbreak occurred in Cameroon where a total of 10,759 cases with 657 deaths were reported. The Far North Region of Cameroon was the epicenter of the outbreak during which almost 9,500 cases with 600 deaths were reported, the most severe outbreaks of cholera during the last fifteen years. This study seeks to understand the spatial and temporal dynamics of the 2010 epidemic in the Far North Region. A Geographic Information System (GIS) and spatial statistics are used to map and explore the spatial and temporal pattern of reported cases in 2010. The results show that the attack rate varied from 4.2 per 100 000 inhabitants in Kaélé health district to 1293.1 per 100 000 inhabitants in Kolofata health district, while the case fatality rate varied from zero in Bourha health district to 66.7 % in Mindif health district. We found that the epidemic broke out with the first rainfall in early May. The epidemic developed mainly during the months of high rainfall, in particular August, that had a monthly mean of 300 mm and 14 days of rain. The epidemic ended with the end of the rainy season in December. With analysis of additional years of data, positive correlation could be further understood between the attack rate and the rainy season. This study reveals that almost all the affected areas lacked access to a good potable water supply.

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HAS IMPROVED WATER AND SANITATION CHANGED THE PREVALENCE OF SCHISTOSOMIASIS AND SOIL TRANSMITTED HELMINTHS (STH) AMONGST PRIMARY SCHOOL AGED CHILDREN IN UGU DISTRICT OF KWAZULU - NATAL

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In South Africa, it is estimated that 2.5 million people are infected with schistosomiasis and a much larger but unknown number with geohelminths, as reported previously. This study is to be conducted in the south coast region of Ugu district in female primary school aged children from schools that are located in rural and semi-rural areas below 3000 meters attitude from sea level. Two common soil transmitted helminths are targeted i.e. *Ascaris lumbricoides* and *Trichuris trichiura* as well as *Schistosomiasis haematobium* (urinary bilharzia). Schools will be selected

randomly from across the region and consent will be obtained from parents and assent from young girls aged 10-12 years. Three urines and one stool sample will be collected per participating individual at the school after demographic information collected from each subject investigated. Samples will be safely transported to the laboratory for microscopic quality controlled analysis by the study PI hence prevalence and intensity will be determined and also whether improved delivery of basic resources to the community has impacted the prevalence and intensity of the targeted parasites. The findings from the study will also be compared with published findings from the piloted study of Parasite Control Programme which was designed in 1997 and targeted three common STHs i.e. *Ascariasis lumbricoides*, *Trichuris trichiura* and hookworm as well as urinary Schistosomiasis haematobium.

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BARRIERS AND MOTIVATORS FOR HANDWASHING AMONG MOTHERS OF NEONATES IN RURAL BANGLADESH

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Infections are important causes of neonatal mortality in Bangladesh. One study observed that maternal handwashing was associated with reduced neonatal mortality. To design a maternal handwashing intervention, we conducted a qualitative study to identify barriers and motivators to handwashing among primiparous mothers of neonates and infants in Matlab, a rural area of Bangladesh. We observed 20 mothers of neonates to understand the contextual factors that facilitated or hampered their handwashing behavior. We conducted indepth interviews with 32 mothers of neonates and infants to explore perceptions, beliefs, and practices related to handwashing. Mothers perceived the need to wash hands with or without soap before eating, or before feeding a child by hand. Mothers reported that elders advised new mothers to wash hands if eating after breastfeeding; mothers believed their child could die if they did not wash their hands after breastfeeding and then ingested their own breastmilk from their hands. Although mothers expressed the importance of washing hands before holding a baby to prevent skin problems and diarrhea, we only occasionally observed them to wash hands before holding their own baby. They prioritized using soap if there was any visible dirt or feces. Otherwise, washing hands with water alone was deemed sufficient. Mothers perceived that infrequent handwashing is a social norm in this rural area. The new responsibilities of nurturing a newborn, who eats and defecates frequently, was cited by mothers as a barrier to washing hands, often because they simply forgot. Sometimes mothers avoided handwashing because they believed neonates could catch a cold if mothers frequently touched water. Reinforcement by family members during the neonatal period and perceived risk of getting diarrheal illness were cited as important motivators for washing hands. Reminders from family members and risk perception are important motivators to maternal handwashing behavior during the neonatal period in rural Bangladesh. Enhancing external cues, by engaging family members and providing visual reminders of critical times for handwashing, leveraging existing cultural beliefs, and clarifying neonatal health threats, may improve maternal handwashing behavior in the neonatal period.

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ISOLATION OF *ESCHERICHIA COLI* 0157:H7 AND OTHER AEROBIC PATHOGENS FROM HAWKED FOODS IN EKPOMA, EDO STATE, NIGERIA

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Food-borne diseases present public health challenges related to food-handling practices. Prompt and thorough laboratory evaluation of cases and suspected foods is essential. This study therefore designed to determine the laboratory evaluation of pathogens from Hawked Foods in Ekpoma, Nigeria This experimental study was carried out at the diagnostic and research laboratory, Ambrose Alli University, Ekpoma. A total of 50 samples were collected randomly from hawked edible foods from different locations in Ekpoma area. The samples were cultured on Eosin Methylene Blue and Sorbitol MacConkey Agar as well as selenite F-Broth, Nutrient Broth and Deoxycholate Citrate Agar. Plates were incubated overnight at 37°C, and bacterial isolates were identified based on Morphological, biochemical and serological characteristics. The samples obtained were placed in a clean polythene bag and immediately transferred to the laboratory and inoculated into appropriate media Glasswares were sterilized in a hot air oven at 160° C for 1 hour before use. Out of 50 samples, 36 (72%) shown a bacterial growth and 14 (28%) shown no bacteria growth. Of this 36 samples showing bacterial growth after 24 hours incubation, 1 (2.8%) yielded *E. coli* 0157:H7, while other strains of *E. coli* accounted for 11 (30.6%), *Klebsiella aerogenes*, 4 (11.1%), *Salmonella* sp, 5 (13.9%), *Proteus* sp, 6 (16.7%), *Shigella* sp, 4 (11.1%), Coliforms 4 (11.1%) and 1(2.8%) yield *Staphylococcus aureus*. The bacterial isolates revealed low prevalence of *E.coli* 0157:H7 in hawked food samples (2.8%) in comparism with other enteropathogens *E. coli* strains (30.6%)), *Klebsiella aerogenes* (11.1%), *Salmonena* sp, (13.9%), *Proteus* sp (16.7%), *Shigella* sp (11.1%), *Staphylococcus aureus* (2.8%) isolated (P<0.05) The study confirmed the presence of *E. coli* 0157:H7 among other enteropathogens isolated from hawked foods items in Ekpoma, Nigeria. Burns, carrot, peas and meat had the highest frequency of isolated organisms while cassava, egg roll, and locus beans has low frequency.

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PERSISTENT OPEN DEFECATION IN BANGLADESH COMMUNITIES DESPITE HIGH PROPORTION OF HOUSEHOLDS WITH LATRINES

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Defecation in an open environment is a risk factor for diarrheal disease. Interventions aimed at reducing environmental fecal contamination prioritize latrine coverage. We analyzed baseline data from a rural population to assess the relationship between the presence of human feces in the environment and access to a latrine. Field workers conducted a cross sectional survey and environmental spot check to collect data on latrine ownership, sanitation practices and observations of visible feces in a sample of 1,431 households in 506 compounds in rural Bangladesh. Even though no latrine facility was reported for 25 (2%) households, human feces were observed inside 174 (12%) households; within 338 (24%) courtyards in the compounds, which serve as a social gathering place for household members; and in 658 (46%) areas surrounding the compounds. Latrines were privately owned in 662 (47%) households. Among these, 81 (12%) had visible feces in the household and 147 (22%) in their courtyards. There was a latrine available for use in 235 (97%) households that had visible feces in the courtyard. Whether or not the family owned or only shared a latrine, they were equally likely to have visible feces at both household and courtyard levels. Thirty two percent of fathers in households who owned a latrine reported not always using the latrine. Visible feces at the compound level was seen less commonly among households with a latrine with indications of regular use, such as a well worn path (OR: 0.53, 95% CI: 0.34-0.71). When asked about the last site of defecation for a child < 3, 69% of mothers reported that they defecated in the courtyard and 91% of these feces were disposed in bushes, drains or left in the open. Mothers from compounds where we observed visible feces reported more often that their children > 5 sometimes used the latrine compared with compounds where mothers reported their children > 5 always used the latrine (OR: 6.1, 95% CI: 3.9-

9.5). Fecal contamination of the rural household environment remains common despite the presence of latrines. Further research to explore why people do not use their latrines could help to develop interventions to encourage consistent use of available latrines for defecation and sanitary child feces disposal to reduce environmental contamination.

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A NEW STRATEGY OF EMERGENCY TREATMENT FOR *SCHISTOSOMA JAPONICUM*-INFESTED WATER

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The purpose of the present study was to investigate the effect of suspension concentrate of niclosamide (SCN) on killing cercaria of *Schistosoma japonicum* that floats on water surface, and its toxicity to fish, so as to establish an emergency-treatment intervention for rapidly killing cercaria and eliminate water infectivity. SCN was formulated into different concentrations of solutions, and then were sprayed on surface of the *S. japonicum* cercaria-infested water. The water infectivity was determined using mice at 0, 10, 30 min after spraying. SCN was formulated into a solution of 100 mg/L, and then were sprayed on surface of the water with niclosamide dosages of 0.01, 0.02, 0.03 and 0.04 g/m2. At 30 and 60 min after spraying, the water infectivity was determined using mice. Zebra fish were transferred into the static water, then 100 mg/L SCN, with niclosamide dosages of 0.01, 0.02, 0.03 and 0.04 g/m^2, were sprayed on water surface. At 0, 10, 30, 60 min after spraying, water samples were collected at water depths of 0, 10, 20, 30, 40 cm, and niclosamide was determined using high-performance liquid chromatography. And the death of zebra fish was continually observed for 96 h after spraying SCN. At 0, 10, 30 min after spraying 1 000, 100, 10, 1, 0.1 mg/L SCN on water surface, the infectivity of water all significantly decreased. Among them, at 30 min after spraying 1 000 mg/L and 100 mg/L SCN, no *S. japonicum* infectivity was detected in water. At 30 min after spraying 100 mg/L SCN, with niclosamide dosages of 0.01, 0.02, 0.03, 0.04 g/m^2, the water infectivity reduced significantly, and no infectivity was found at 60 min after spraying SCN. The surface of static water was sprayed with 100 mg/L SCN, the peak concentration was found at 0 min, and the solution diffused to site with a water depth of 10 cm after 10 min. 30 min later, SCN diffused to the whole water body, and distributed evenly. After spraying 100 mg/L SCN on surface of water with a volume of(3.14×202×50)cm^3, with niclosamide dosage of 0.02 g/m2, 96 h later, no death of zebra fish was found. It is concluded that spraying 100 mg/L SCN, with niclosamide dosage of 0.02 g/m^2 on surface of *S. japonicum*-infested water, the water infectivity can be eliminated after 30-60 min, and there is no evident toxicity to fish. This cercaria-killing method, as an emergency-treatment intervention for infested water, can be applied in those surveillance and forecast system for schistosomiasis.

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BOILING DRINKING WATER IN PERI-URBAN ZAMBIA: A COSTLY AND INEFFECTIVE APPROACH TO IMPROVE MICROBIOLOGICAL QUALITY

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Unsafe drinking water is a leading cause of preventable diarrheal disease, particularly among children in developing countries. Waterborne pathogens contribute to an estimated 4 billion cases and 2.5 million deaths from diarrheal disease each year. It is estimated that almost 900 million people lack access to improved drinking water worldwide; over 5 million of those live in Zambia. For those without access to reticulated water supplies, boiling is the most common method of disinfecting water in the home and the benchmark against which other point-of-use water treatment is compared. In a five-week study in peri-urban Zambia, we assessed the microbiological effectiveness and cost of boiling among 49 households without a water connection who reported "always" or "almost always" boiling their water before drinking it. Source and household drinking water samples were compared weekly for thermotolerant coliforms (TTC), an indicator of fecal contamination. Demographics, costs and other information were collected through surveys and structured observations. Drinking water samples (geometric mean 7.2 TTC/100ml, 95%CI 5.4-9.7) were actually worse in microbiological quality than source water (geometric mean 4.0 TTC/100ml, 95%CI 3.1-5.1) ($p<0.001$), although both are relatively low levels of contamination. Evidence suggests that water quality deteriorated after boiling due to lack of residual protection and unsafe storage and handling. We found that using a drinking cup to transfer freshly boiled water into the storage container was strongly associated with a decline in drinking water quality ($p<0.01$). The constructed cost of solid fuel or electricity used for boiling was estimated to represent a median 5-7% of income. In this setting where microbiological water quality was relatively good at the source, safe-storage practices that minimize recontamination may be more effective in managing the risk of disease from drinking water at a fraction of the cost of boiling.

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MICROBIOLOGICAL EFFECTIVENESS OF TREATING AND SAFELY STORING SHALLOW TUBE WELL WATER IN RURAL BANGLADESH: A PILOT STUDY

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Chlorine treatment and safe storage can improve microbiological quality of drinking water and reduce diarrhea. In Bangladesh, where the population mostly uses tubewells, the effectiveness of chlorine may be compromised by groundwater constituents that exert chlorine demand, leading to low chlorine residual. Moreover, fecal contamination in tubewells is sporadic and at moderate levels, suggesting safe storage to prevent contamination at the point-of-use may suffice to ensure microbiologically safe drinking water. We conducted a pilot study in rural Bangladesh to assess the microbiological field effectiveness of sodium dichloroisocyanurate (NaDCC) tablets and two types of storage containers. We enrolled 80 households using tubewell water with no self-reported presence of iron, as chlorine demand by iron adversely affects disinfection. Half of the households were randomized to receive a 10-liter jerry can or storage jar with lid and tap. In addition, half were randomized to receive NaDCC tablets and the rest received no water treatment. We assessed the microbiological quality of tubewell and stored water by H2S testing for all 80 households six weeks after we distributed the products, as well as by membrane filtration for Escherichia coli in 24 households three and six weeks after product distribution. At the same two time points, we measured free chlorine in stored water by digital colorimeter in the 40 households receiving tablets. Of 24 tubewells tested by membrane filtration, E. coli in moderate concentrations (range of 1 to 31 CFU/100 mL) was detected in 12.5% and 25% of the wells at the two subsequent testing points. H2S test was positive in 30% of 80 wells. Among households not receiving tablets, 7% of jerry can samples and 19% of storage jar samples coming from uncontaminated tubewells had a positive H2S test, suggesting within-household contamination. H2S testing showed no contamination in stored water treated with chlorine. Free chlorine was above the CDC recommendation of 0.2 mg/L in 85% of samples after three weeks and 90% after six weeks. NaDCC tablets provided sufficient chlorine residual and improved microbiological water quality. Chlorine treatment coupled

with safe storage was more effective at reducing contamination than safe storage alone, while of the two containers, the jerry can was less frequently contaminated.

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IMPACT OF A SAFE WATER AND HYGIENE PROGRAM IN RURAL HEALTH FACILITIES, ZAMBIA, 2010

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In developing countries, many health facilities lack access to safe water for drinking and handwashing, putting patients and health workers at risk for health facility-acquired infections. In Zambia, where only 48% of the rural population has access to improved water supplies, we installed handwashing and drinking water stations (40-liter plastic buckets with spigots and lids, metal stands, chlorine solution, and soap) in eight rural health facilities, conducted training in water treatment and storage, and hand washing, and evaluated the impact on health worker and patient knowledge and practices. In February 2010, we conducted baseline surveys of health worker and patient knowledge and practices regarding handwashing, safe water storage, and water treatment in eight health facilities, tested stored water in clinics and households for residual chlorine as an objective measure of water treatment, and observed hand washing technique among patients. In March 2010, we installed handwashing and drinking water stations in the health facilities and trained health facility staff. In July 2010, we conducted a follow-up evaluation using the baseline survey instruments. Chlorination of stored water was observed in 0 clinics at baseline and four (50%) at follow-up; seven (88%) clinics were using the installed water stations at follow-up. Compared to baseline, a higher percentage of patients at follow-up used improved water storage containers at home (24% vs. 61% [p<0.001]), had detectable residual chlorine in stored water (3% vs. 15%, p=0.025), and demonstrated correct handwashing procedure (42% vs. 65% [p=0.016]). Installation of water stations combined with training in rural Zambian clinics resulted in improved water treatment and storage practices in health clinics and patients, and increased ability to demonstrate proper handwashing technique among patients. This intervention has now expanded to 150 additional health facilities in Zambia.

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RESPONDING TO AN OUTBREAK OF TYPHOID FEVER: ASSESSMENT OF WATER, SANITATION, AND HYGIENE INTERVENTIONS IN NENO DISTRICT, MALAWI

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Typhoid fever, caused by *Salmonella enterica* serovar Typhi, results in an estimated 21 million cases and 200,000 deaths annually worldwide. On May 2, 2009 an outbreak of typhoid fever began in rural villages along the Malawi-Mozambique border. Despite numerous interventions, including distribution of WaterGuard (WG) for in-home water treatment, cases of typhoid fever continued, resulting in 748 illnesses and 44 deaths by September 2010. To better understand knowledge, attitudes, and practices surrounding typhoid fever, safe water, and hygiene, and to inform future interventions, a survey was administered in September, 2010, to female heads of randomly selected households in 17 villages in Neno District, Malawi and stored household drinking water was tested for free residual chlorine. Among 202 households, primary sources of drinking water were boreholes (48%), unimproved wells (46%) and rivers (4%). Households who previously attended a community-wide typhoid talk were more likely to report that typhoid fever is caused by poor hygiene and (85% vs. 64%, P = <0.01) drinking unsafe water (55% vs. 42%, P = 0.02) compared to households not attending a talk. WG was present in 53% of households; only 33% of those households reported treatment of currently stored water. Residual free chlorine levels were adequate in stored water samples from only 15% of all households surveyed, but were adequate in 63% of households that reported water treatment and had WG present. Seventy-seven percent reported soap in the home, but only 46% reported use of soap for hand washing. Knowledge regarding the association between unsafe drinking water and typhoid fever improved after community-wide education, but remains low. Despite the presence of WG in over half of all households, less than one-third were using this method to protect their drinking water. Therefore, many households remain without safe drinking water. Future interventions should focus on increasing use of WG and improving hand washing and hygiene practices to prevent waterborne illnesses, including typhoid fever.

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DETECTION OF *ANAPLASMA PHAGOCYTOPHILUM* INFECTIONS: A CASE SERIES FROM A SUBURBAN COMMUNITY HOSPITAL IN MASSACHUSETTS

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Human granulocytic anaplasmosis (HGA) can range in presentation from subclinical disease to severe febrile illness and death. We examined medical records for patients diagnosed with HGA at Newton-Wellesley Hospital in 2009, and compared rates of infection to confirmed cases of HGA in Massachusetts. Epidemiologic case confirmation requires both laboratory confirmation of disease and a corresponding clinical history obtained from a clinician or the patient. A PCR tick-associated pathogen panel that included assays for *Anaplasma phagocytophilum, Ehrlichia chaffeensis, E. ewingii*, *Borrelia burgdorferi* and *Babesia microti* was used for laboratory diagnosis. Thirty-three cases of *A. phagocytophilum* were confirmed during 2009 at our hospital. Thrombocytopenia and/or leukopenia were observed at the time of presentation in 30/33 (91%) patients, and 28/33 (85%) reported fever. Cases were geographically distributed diffusely throughout the hospital catchment area in 21 zip code regions, with the exception of one cluster of seven cases in a single zip code area. Because few clinicians indicated suspicion for non-Lyme tick-borne disease, the use of a tick panel that includes PCR testing for several organisms could improve disease detection of underrecognized tick-borne diseases. In Massachusetts, the Department of Public Health estimates of confirmed HGA cases demonstrate a steady increase since 2005 (23 cases compared to 61 in 2010). Of the 33 cases our hospital reported to the MDPH in 2009, only 20 (62%) were confirmed due to a lack of accompanying clinical data. Statewide in 2009, only 61/146 (42%) of reported PCR positive HGA results became confirmed cases, primarily due to absence of reporting clinical data to the MDPH. Lack of communication between clinicians and public health authorities, in addition to scarce resources for epidemiologic follow up, prevent accurate case reporting and assessment of the true HGA burden.

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IMPACT OF CONFIRMED INVASIVE BACTERIAL INFECTION IN THE CHILDREN MORTALITY IN PEDIATRIC CHU-GABRIEL TOURÉ

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The new tendencies and characteristic of children mortality are function of the sanitary arrangements, environmental and socio-economic conditions which prevail in a population. In the world, the frequency of various pathologies varies according to countries. It's in developing countries such as African's that the high probability of death is notified. Invasive bacterial infections are responsible of serious pathologies such as meningitis, bronchopneumonia, septicemias, peritonitis, typhoid fevers and especially diarrheas which constitute a handicap for the infantile population in developing countries. To study the etiologies of children mortality, systematic blood smears for malaria parasites were integrated into ongoing hospital-based surveillance for invasive bacterial infection at CHU Gabriel Touré in Bamako, Mali. Children aged 0-15 years with fever ≥ 39° C or suspicion of invasive bacterial infection (SIBI) admitted to HGT were eligible. Blood and relevant body fluids were collected and cultured after obtaining informed consent. Blood smears for malaria were performed. Cases are define as children included in the study and died during follow up. From January 2008 to December 2010, 15278 children were included. Among this, 1169 died (7, 65%). Pathogens have been isolated in 433 samples among whom 270 (62%) was invasive bacterial infection and 163 (38%)was malaria. The mean age was 30,51 ±40,36. The most frequent pathogens in blood culture was *Streptococcus pneumoniae* (80/232), *Escherichia coli* (22/232), *Staphylococcus aureus* (23/232). Children less than 1 year was the most affected (115/232, $p = 10^{-3}$). In Cerebrospinal fluid, the isolated pathogens were respectively: *S. pneumoniae* (61/101), *Hæmophilus influenzae* b (11/101) and *Neisseria meningitidis* (10/101). No significant differences have been observed between the three years but we noticed two peaks in April and October every year. In conclusion, children less than 1 year are highly affected by childhood mortality. In most of the case any pathogen is incriminated. Gram negative bacilli and S. pneumoniae are responsible of the greatest rate of mortality. Efforts should be done to improve the identification and prevention of pathogens responsible of child mortality.

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A NEW APPROACH TO THE MANAGEMENT OF SEVERE ANAEMIA IN *PLASMODIUM FALCIPARUM* INFECTION

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Rupture of invaded Red blood cells as they release merozoites into the blood circulation, is a cause of the anaemia in *Plasmodium falciparum* Infection. There appears to be yet another and, perhaps, more serious mechanism that contributes to the severe anaemia seen in *P. falciparum* infection. Some patients on blood transfusion (whole blood or packed cells) for severe anaemia in *P. falciparum* infection have been observed to return to square one (became pale again) within 24 to 72 hours of such transfusion. Giving more blood never changed the situation as they always returned to square one. The issue of jaundice seen in some of these cases tends to start when the spleen began to enlarge and did not correspond to the degree of anaemia as it was usually mild. In some of these patients, there was no jaundice, the level of bilirubin in the blood was normal and there was no urobilirubin in the urine. Perhaps the severe anaemia in *P. falciparum* infection is due to a phenomenon of massive pooling of un-invaded red blood cells from the peripheral circulation into some capillary beds ?the liver and/or the intestine. This may be an auto-protective mechanism to prevent these cells from being invaded by the *P. falciparum* merocites as they are released into the circulation from the liver. The anaemia in all these cases of severe anaemia that returned to square one after blood transfusion was corrected by adequately treating the malaria and reversing the Auto-Protective massive pooling of un-invaded red blood cells from the peripheral circulation, without further blood transfusion. Perhaps the solution to the management of severe anaemia in *P. falciparum* Infection is not Blood Transfusion but adequate treatment of the malaria fever {total clearance of malaria parasites} and the reversal of the auto-protective massive pooling of un-invaded red blood cells from the peripheral circulation phenomenon.

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HELMINTH ROLE IN LOWERING THE ATHEROSCLEROSIS RISK FACTORS: EVIDENCE IN A POPULATION AT SECONDARY EPIDEMIOLOGICAL TRANSITION

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Atherosclerosis is characterized by chronic local inflammation of the vascular wall, involving both innate and adaptive arms of immunity and is largely due to combination of inflammation and dyslipidemia. It is known that chronic helminth infections are associated with lower nutritional status and anti-inflammatory response and a reduction of helminth burden might therefore play a role in the development of cardiovascular disease in developed societies. We aim to investigate the association of helminthes and atherosclerosis in a population in transition. In Flores, Indonesia, an area highly endemic for geohelminthes, a cross-sectional study was performed in 1040 participants. Stool samples were collected and tested for *Ascaris lumbricoides* and *Necator americanus* by PCR and *Trichuris trichiura* by microscopy. A subset of 528 adults aged 40-80 (male/female) was included for intima media thickness (IMT) measurement. In addition we also measured information on atherosclerosis risk factors such as blood pressure, body mass index (BMI), waist hip ratio (WHR), lipid level, blood glucose (FBG), CRP and whole blood stimulated cytokine production. The classical cardiovascular risk factors such as WHR, BP, FBG, CRP, triglyceride, and ratio of TC/HDL-c were positively associated with IMT while innate stimulation of IL-10 by LPS were negatively associated with IMT. Helminth infection was negatively associated with BMI, WHR, lipid levels, blood glucose, and CRP, but we found no direct association of helminths and IMT. The atherosclerosis is a process that progresses over a lifetime. We have shown that current helminth infections were not directly reducing the risk of atherosclerosis, but were involved in lowering its risk factors. Therefore, we conclude that a reduction of helminth burden might play a role in the rise of cardiovascular disease in developed societies.

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DESCRIPTIVE STUDY OF IRON BIOMARKERS IN ETHIOPIAN VISCERAL LEISHMANIASIS PATIENTS

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Visceral leishmaniasis (VL) is a neglected systemic parasitic disease caused by the *Leishmania donovani* complex species. It commonly affects poor populations in the tropics and sub-tropical endemic areas, causing 500,000 morbidities and more than 50,000 deaths annually. Although anaemia is a common sequel of VL, use of iron status biomarkers in

these patients is not well studied. This study was undertaken to describe the clinical characteristics, and changes in iron status biomarkers (ferritin, sTfR, and hepcidin) at admission and during a month following commencement of anti-leishmanial treatment in newly diagnosed VL patients. A prospective longitudinal descriptive study was conducted in a newly diagnosed, HIV negative VL patients admitted to Arba Minch Hospital-Leishmaniasis Research and Treatment Centre, South-West Ethiopia, between April and May 2010. A total of 20 confirmed VL cases, 2 female and 18 male, with a median age of 18 years were included in the study. While fever was the initial presenting symptom, with mean duration of 4.4±3.7 months, 6 (30%) patients had no measurable fever despite repeated follow-ups. Splenomegaly was present in all patients with 12 (60%) of them being malnourished. Pancytopenia was a common hematologic manifestation. The Mean±SD of haemoglobin at admission was 7.2± 1.99g/dl with 9(45%) of patients being iron deficient (ID). Ferritin was elevated at baseline, 1373.13±1191.19µg/l, which significantly decreased following anti-leishmanial treatment. sTfR was increased in ID patients; and serum hepcidin concentration was higher in non-ID (NID) patients. Significant correlation ($p<0.05$) observed between sTfR and haemoglobin, hepcidin and ferritin, ferritin and body mass index, and sTfR and sTfR-F index. With treatment, significant improvement was observed in both clinical and laboratory parameters. In conclusion, TfR-F index was a useful biomarker in differentiating ID and NID patients. Iron deficiency contributed to development of anaemia in about half of the patients. A future study is recommended to evaluate utility of serum sTfR and hepcidin against bone marrow staining for iron, and consideration of iron intervention efficacy in ID patients

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UTILIZATION OF ARTEMISININ-BASED COMBINATION THERAPY AMONG CHILDREN UNDER FIVE YEARS OF AGE WITH SUSPECTED MALARIA IN JINJA DISTRICT, UGANDA

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In 2004, the Ugandan Ministry of Health (MoH) adopted artemisinin-based combination therapy (ACTs) for treatment of uncomplicated malaria. Artemether-lumefantrine (AL) and artesunate-amodiaquine were recommended as first line and alternative treatment, respectively. However, policy implementation has been slowly translated into practice. This study sought to establish the proportion of children under five with suspected malaria treated with ACT and factors associated with ACT use in Jinja district, a peri-urban area with mesoendemic malaria transmission. Two-stage cluster sampling was used to identify eligible households. Caretakers (n=380) of children under five who had suspected malaria four weeks preceding the study date were interviewed by trained study personnel using pretested standardized questionnaires. Suspected malaria was defined as any illness in a child < 5 years of age perceived as malaria by the caretaker irrespective of whether this was confirmed or not. Antimalarials administered were determined by the caretakers' report. If an ACT was reported, the regimen was verified using sample drugs and packages. A child was considered to have received an ACT if they had received any ACT regimen, at any dose, for any duration. Out of 380 children studied, 207 (54.5%) had received an ACT; AL was the only ACT administered. There was significant association between utilization of AL and source of treatment (OR=16.35, 95% CI 9.00-29.71), caretaker's knowledge about first line treatment for uncomplicated malaria (OR=2.15, 95% CI 1.23-3.76) and whether the caretaker had heard about ACTs before (OR=3.07, 95% CI 1.40-6.70). Out of 207 children that had received AL, most (79.7%) had acquired it from public health facilities. Children who did not receive an ACT had most commonly visited drug shops (52.6%) and private clinics (27.2%). Out of 173 caretakers whose children had not received ACT, the reasons reported included: not prescribed by the health worker (49.7%), out-of-stock (17.3%), and unknown (16.2%). Only 2.9% of caretakers reported that AL was too expensive. ACT utilization among children with malaria in Jinja, Uganda is far below the national malaria control target of 85%. Source of treatment and caretaker knowledge about ACTs impact on the utilization of ACTs. There is need for subsidized ACTs to be made available through private sector, including drug shops, where many children access care but are not receiving ACTs.

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PANDEMIC H1N1 2009 SEVERITY IS ASSOCIATED WITH NK AND T CELLS COUNT AND DIFFERENTIATION STATUS

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An antigenically novel H1N1 virus was transmitted to humans from pigs in 2009. It is now known that while infection rates reached pandemic proportions, most cases were mild or asymptomatic. However, at least 50% of severe patients were previously healthy young adults. It is not clear whether this reflects intrinsic viral factors or host immunity. To investigate the contribution of the host immune response we compared lymphocyte counts and phenotypes during the course of acute H1N1 2009 infection in patients with mild and severe illness. Patients with pandemic H1N1 illness confirmed by RT-PCR were enrolled. Fresh peripheral blood samples were assessed by 6 colour flow cytometry using Trucount tubes with panels of monoclonal antibodies to determine absolute counts for lymphocyte subsets and the percentage of CD4, CD8 T cells expressing activation and differentiation markers. Patients were categorized as having severe or mild illness on the basis of clinical findings.62 patients were enrolled including 51 with mild and 11 with severe illness, of which 2 died. The age and sex of severe and mild patients was similar but severe patients admitted later and were hospitalized longer. Lymphopenia was more common in severe cases with CD4 lymphopenia in 60% of severe versus 15% of mild patients ($p=0.007$). CD4:CD8 ratios were also decreased. NK cell counts were lower in severe patients on all illness days and this was significant on day 7 ($p = 0.002$). B cells counts were similar. CD8 T cell activation and differentiation tended to be rapid in patient with mild illness but delayed and exacerbated in those with severe illness with accummulation of the CD27+CD28- subset.This study shows that pandemic H1N1 severity is associated with low NK and CD4 T cell counts. NK cells and other pre-existing effector mechanisms are likely to be important for preventing severe influenza because virus titers generally peak early after infection. Lymphopenia and inverted CD4:CD8 ratios are also common in highly pathogenic H5N1 infection indicating that lymphopenia is associated with severe illness irrespective of the virus strain. We suggest that CD4 lymphopenia and aberrant CD8 activation are consequences of excessive virus growth which can occur when infected with a highly virulent virus or with a low virulence virus when early immune responses are inadequate.

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IN-HOSPITAL PREVALENCE AND PROGNOSIS OF NEONATAL TETANUS IN MBUJIMAYI, DEMOCRATIC REPUBLIC OF CONGO

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Most of 1 million yearly deaths from tetanus occur in Sub-saharian Africa and 90% of them corcern newborns(WHO report).In our circle, 3 previous studies on neonatal tetanus were undertaken and reported the prevalence of 0.79% in 2003; 0.85% in 2005 and 2.10% in 2007.In

order to determine the prevalence of neonatal tetanus and epidemiologic-clinical factors that influence its evolutionary prognosis in term of mortality,a retrospective study based on analysis of hospital documents and spread out over 36 months was conducted last year(2010) in the Pediatric Departement of Bonzola Hospital at Mbujimayi and related to 114 tetanic newborns admitted during the period of 2007-2009. The prevalence of neonatal tetanus was 2.18% with about 3 new cases by month.Of overall 114 cases, 77 newborns(67.54%) died and the majority of them died in 1-3 days after admission.There was no significant correlation between sex and mortality(sex ratio was 1.5).At admission, 22 newborns(19.30%) had a tetanus of class II and 92(80.70%) a tetanus of class III according to the International Classification of Dakar.The majority of tetanic newborns(70.17%) came from the vicinity of Mbujimayi and all of them were born at home.Only 19 mothers (16.67%) respected calendar vaccination.The mortality rate was influenced by:absence of pregnent women's vaccination(83.33%);entry door of tetanus(umbilical in 82.46% of the cases);method of umbilical cord section(razor blade with 80.70% against pair of scissors with 12.42%) and the severity of the disease at admission(tetanus of class III with 80.70%).Neonatal tetanus is still a serious problem of Public Health in Mbujmayi,so that measures active on factors above-mentionned are required in order to improve this situation.

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DIFFERENT PHARMACOKINETIC APPROACHES WHEN ANALYSING ARTEMETHER AND DIHYDROARTEMISININ IN PREGNANT WOMEN WITH UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN UGANDA

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Pregnancy alters the pharmacokinetic (PK) properties of many antimalarial compounds what might result in lower drug exposure and increased risk of treatment failure. Therefore, the pharmacokinetic properties of antimalarial compounds need to be characterized in specific subpopulations. The objective of this study was to evaluate the PK properties of artemether (ARM) and its metabolite dihydroartemisinin (DHA) in pregnant women with uncomplicated *Plasmodium falciparum* malaria in Uganda. Non compartmental analysis (NCA) was performed using WinNonlin and compared to parameter estimates obtained by population PK modeling using NONMEM. A sequential and a simultaneous 1 compartment (CMT) ARM, 1 CMT DHA with 1st order absorption and elimination population PK model was used in the comparison. The simultaneous ARM-DHA model was further developed by the evaluation of different distribution, absorption, error and covariate models. A simultaneous model with sequential zero-order and transit-absorption and a 1 CMT distribution model for ARM and DHA provided the best fit to the data. Using NCA or sequential drug-metabolite modeling, the metabolite was assumed to be absorbed from the gut into the systemic circulation however this is mechanistically incorrect. In a simultaneous population PK drug-metabolite model the absorption and the *in vivo* conversion of the parent drug into the metabolite dictates the appearance rate of the metabolite. This enables an understanding and a PK interpretation of different drug exposure obtained in different populations. The different PK approaches resulted in large differences (5 to 7-fold) in the estimated apparent volume of distribution, which emphasize the need for using an adequate analysis approach. This has to be considered when comparing different methodologies used in available published literature. In conclusion, the population PK properties of ARM and DHA were described by a simultaneous model in pregnant women with malaria. Simultaneous population PK models should be used in the analysis of drug-metabolite data to be able to obtain parameter estimates that reflect physiological values.

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NEW VACCINATION DELIVERY REGIMEN DRIVES ENHANCED VACCINE-SPECIFIC IMMUNE RESPONSES

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The goal of vaccine delivery is to present vaccine immunogens/organisms in manner that enhances antigen presenting cell activation, uptake of antigen and processing. We focused on use of a gel slurry delivery method to drive pro-inflammatory, vaccine-specific responses with or without the use of CpGs as adjuvant. The gel slurry hardens at body temperature, forming a gel matrix depot that releases CpGs and antigen, attracting antigen presenting cells. Using recombinant hepatitis B antigen, we evaluated 8 different vaccine delivery schemes ability to induce antibodies and cytokines to recHepB ag. Mice were vaccinated with recHepB ag in two different gel slurrys, with Alhydrogel (Alum) or with Complete Freunds adjuvant (CFA). The slurry and alhydrogel delivery methods were +/- CpGs. Initial results showed mice vaccinated with either gel slurry plus CpGs had significantly higher vaccine-specific IgG2a 14 days after the prime, and IgA, IgM at 28 days post inoculation than mice vaccinated with alum or CFA. One gel slurry delivery drove significantly higher vaccine-specific IgG titers 14 days post-prime, than the other delivery methods did post-boost. Suggesting that the boost was unnecessary. Recall assays showed upregulated IL-10 and IL-4 from splenocytes of mice vaccinated with Alhydrogel or CFA compared to cells from gel-slurry + CpG vaccinated mice. CpG use reduced levels of IL-5 to background in all groups compared to elevated levels in CFA. No differences in levels of IFNγ or TNF were seen. Based on the data that showed a Th1 driven immune response after prime inoculation, we are currently assaying for T cells activation with flow cytometry at 28 days. The use of gel slurry plus CpGs, or other pro-inflammatory adjuvants to deliver vaccine antigens/organisms could have great utility for enhancing vaccines such as influenza, hepatitis A and B, as well as in development of vaccines for parasitic diseases. Ultimately, this type of vaccine delivery system may facilitate development of therapeutic vaccines as well as prophylactic.

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UNDERSTANDING THE CHALLENGES FOR ELIMINATING TUBERCULOSIS FROM TROPICAL COUNTRIES

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Tuberculosis (TB) poses a serious threat to public health in low-income tropical countries. Substantial variability in the response to antituberculous therapy and prolonged duration of infectivity in many of these patients provide challenges for elimination of tuberculosis in tropics. Within this background we conducted a hospital based descriptive study over the period of 2 years (Jan 2009 to Dec 2010) in the department of internal medicine at B.P Koirala Institute of Health Sciences, a university teaching hospital in eastern Nepal to characterize the factors responsible for prolonged infectivity in smear positive pulmonary tuberculosis patients. 150 consecutive patients with smear positive pulmonary tuberculosis were included in our study. Patients that remained smear positive even after 2 month of Antitubercular treatment were considered as having prolonged infectivity. All the socio-demographic predictors for tubercular infection, disease and affecting prolonged infectivity based upon clinical experience, published literature and biological plausibility were recorded and subjected to univariate and multivariate analysis for significance testing. Our study revealed striking dose-response relationship between tobacco smoking as well as exposure to indoor air pollution and prolonged infectivity. Past history of pulmonary tuberculosis, HIV seropositivity, diabetes, high bacteriological burden at treatment initiation and radiological abnormalities especially bilateral infiltrations on initial CXR were also independent predictor of prolonged infectivity. Progress towards

elimination of tuberculosis will remain elusive in tropical countries unless these critical issues are identified; understood and targeted interventions aiming them are formulated. This mandates further discussion of the current TB treatment, control and elimination strategies in tropical countries.

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INCIDENCE OF AND MORTALITY DUE TO SEPSIS AMONG CHILDREN OF 0-5 YEARS HOSPITALIZED AT DIPUMBA GENERAL HOSPITAL

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In order to understand and improve case management of pediatric sepsis, we explored the patterns of incidence and mortality due to sepsis in a pediatric population in Eastern Kasai province (Congo). We reviewed medical records of all 0-5 year children hospitalized at Dipumba General Hospital in Mbuji Mayi over a one-year period. To be included, a case had to be a 0-5 year old child hospitalized and then discharged any time between July 2009 and June 2010, with a documented positive sepsis diagnosis. We reviewed 1482 medical charts and collected data on diagnosis, gender, age, disease onset, admission timing and treatment outcomes. We found 469 cases of sepsis (31.6% incidence rate); these included 263 boys and 206 girls (1 to 1.3 sex ratio). There was no significant difference according to gender. However sepsis cases were significantly more frequent (65.03%) among toddlers (< 3 years) than among older children. We recorded 260 deaths (55,4%). This is significantly higher level of mortality, particularly among cases that were not more promptly brought to the hospital. There is a need to increase the survival rate of children affected with sepsis, notably by improving access to medical care.

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IMMUNOLOGICAL AND VIRAL DETERMINANTS OF DENGUE SEVERITY IN HOSPITALIZED ADULTS IN HANOI, VIETNAM

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Dengue is estimated to affect 50 million people each year and can occur as explosive outbreaks that overwhelm health systems. The emerging picture is that multiple factors including prior immunity, viral load, age of the patient and infecting serotype and genotype may contribute to the severity of dengue infection. This cross-sectional study examined these interactions in adults hospitalized with dengue in low transmission settings, to better identify factors associated with severity across serotype and immunity groups. Patients admitted to the National Hospital of Tropical Disease, Vietnam with a clinical diagnosis of dengue according to the WHO criteria. A patient is considered to have confirmed dengue if either RT-PCR or NS1 is positive, if there is an increase in the level of IgM detected by ELISA or an IgG ELISA conversion in the presence of a positive IgM ELISA. Patients were then categorized as having primary or secondary infection on the basis of serology results. 158 adult patients were enrolled with 130 (82%) laboratory-confirmed cases. Serology was indicative of secondary and primary infection in 61% and 34%, respectively. The infecting serotype was DENV-1 in 42 (32%), DENV-2 in 38 (30%) and unknown in 49 (38%). Secondary infection was significantly more common in DENV-2 patients (79%) compared to DENV-1 patients (36%, $p < 0.001$). This could reflect viral loads which were lower for DENV-2 than for DENV-1 infections but higher in secondary than primary infection. The time until NS1 and plasma viral RNA were undetectable was shorter for DENV-2 compared to DENV-1 ($p \leq 0.001$) and plasma viral RNA concentration on day 5 was higher for DENV-1 (p=0.03). Dengue is emerging as a major public health problem in Hanoi with high rates of primary infection compared to Southern Vietnam and other hyper-endemic regions. DENV-1 and DENV-2 were the prevalent serotypes with similar numbers and clinical presentation but secondary infection may be more common amongst DENV-2 than DENV-1, indicate an association between secondary infection and clinically overt DENV-2 infection. Our study also suggests that primary DENV-2 infections may be less virulent than DENV-1 during primary infection. The situation in Ha Noi provides an opportunity to further examine the roles of serotype and infection sequence in dengue severity and emergence.

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EPIDEMIOLOGICAL VIGILANCE OF *LEPTOSPIRA INTERROGANS* IN PORCINE FARMS THE MEDIO SINÚ IN THE DEPARTMENT OF C'ORDOBA (COLOMBIA)

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Leptospirosis is a re-emerging world wide zoonosis caused for pathogenic spirochetes of the genus *Leptospira*. This disease has been traditionally catalogued as an occupational disease; its presentation is related to a series of epidemiological factors that highlight the presence of animals as canine, rodents and other species of domestic animals or with that one coexists in porcine farms, being of importance the sanitary aspect, the quality of the water and the hygienic of porcine farms conditions. This study was undertaken to realize an epidemiological vigilance in farm workers, canine, porcine and water associated with pig farms in the department of Córdoba. By convenience sampling, in 18 farms, samples of canine and porcine serum and urine, farm worker serum and served and not served water were taken. MAT using 14 serovars of pathogenic *Leptospira* was carried out. Urine and water samples were cultivated in EMJH enriched with 1% rabbit serum. The isolations were confirmed for pathogenic strains by PCR. An inquiry was realized to register general, epidemiological information and pathological precedents. A seroprevalence of 46,66% in pigs, 37% in canine and 77,04% in human beings of these farms was established. Seven *Leptospira* pathogenic strains were isolated from 3 samples of pig urine, 2 samples of canine urine, 1 sample of served water and 1 sample of non served water. In one farm was isolated pathogenic *Leptospira* from samples of porks, canine and water. In conclusion, the maintenance-host role of porcine and canine was demonstrated by leptospiruria, the served waters as propagators of this etiologic agent, and its contribution to the permanency in the environment (non served waters), factors that contribute to the high prevalence of the disease in the workers. The absence of symptomatology, in the human population, compatible with a classic picture of Leptospirosis (jaundice, renal and hepatic failure) confirms that the zone is highly endemic, associated with asymptomatic infections or non-jaundice forms, characterized by general and unspecific symptoms.

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RELATIONSHIP BETWEEN BUILDING MATERIALS AND THE PRESENCE OF VECTORS IN CAJAMARCA, PERU

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We conducted a cross sectional entomological survey for Chagas disease vectors in five communities in the Cajamarca region of northern Peru. Intestinal contents of triatomines were examined for the presence of *Trypanosoma cruzi*. A total of 213 houses were searched for the triatomine vector for one person hour. Eighty-five of 213 household (39.9%) were found to be infested with triatomine insects; all insects were identified as *Panstrongylus herreri* (aka *P. lignarius*). In 38.5 % of infested households at least one insect was found to be carrying the parasite *T. cruzi*. Vector infestation was strongly associated with the housing materials, especially adobe(ODD ratio 5.37, and p-value<0.001) as well as the presence of guinea pigs. In contemporaneous cross-sectional serological surveys 83 of 529 (15.7%) people were found to be seropositive for *T. cruzi* infestation. Although it is possible that additional

vectors are involved in the transmission, the predominance of domiciliated *P. herreri* suggests that this vector is the mediating transmission resulting in some of the highest rates of *T. cruzi* infection in Peru.

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AGGREGATE ORGAN DYSFUNCTION PREDICTS IN-HOSPITAL MORTALITY FROM SEPSIS IN UGANDA

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Sepsis syndrome is not fully characterized in sub-Saharan Africa. We evaluated the association between severity of sepsis and in-hospital mortality in 150 patients with non-surgical sepsis at a regional referral hospital in Mbarara, Uganda. Patients were included if they were ≥ 18 years of age, admitted to the medical ward and had: 1) a suspected infection, and 2) ≥ 2 of the systemic inflammatory response syndrome (SIRS) criteria (temperature >38°C or <36°C; heart rate >90 beats/min; or respiratory rate >20 breaths/min). The patients were predominantly young men (63%) of Nkole ethnicity who were HIV-infected (74%). The mean (± SD) age was 35 ± 14 years. A majority of patients, 120 of 148 (81%), met 3 or 4 SIRS criteria. Sepsis, severe sepsis, and septic shock was diagnosed in 52 (35%), 71 (47%), and 27 (18%) of 150 patients respectively. Of the 98 patients with end-organ dysfunction, 47 (31%) had single organ dysfunction, 36 (24%) had 2 organ dysfunction, and 15 (10%) had 3 or 4 organ dysfunction. In-hospital mortality occurred in 45 of 150 (30%) enrolled patients and in 5 of 52 (9.6%) patients with sepsis, 24 of 71 (33.8%) patients with severe sepsis, and 16 of 27 (59.3%) patients with septic shock. In the multivariate analysis, the identification of severe sepsis (adjusted hazard ratio [AHR] 2.9, 95 % confidence interval [CI] 1.0-8.2, $p = 0.04$), septic shock (AHR 5.7, 95% CI 1.6-20.3, $p = 0.007$) and the dysfunction of 3 or more organs (AHR 2.9, 95% CI 1.1-7.3, $p = 0.03$) increased the risk of in-hospital mortality. Adding aggregate organ dysfunction to the multivariate equation that included sepsis category statistically significantly improved the model but the converse did not (change from previous step, $\chi^2 = 9.7$, $p = 0.008$ vs. $\chi^2 = 5.4$, $p = 0.07$). In conclusion, predictors of mortality were easily measurable and could be used to risk-stratify critically ill patients in resource constrained settings.

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DYNAMIC CHANGES OF 14-3-3ß IN PATIENTS AND MICE INFECTED WITH EOSINOPHILIC MENINGITIS CAUSED BY *ANGIOSTRONGYLUS CANTONENSIS*

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The 14-3-3β protein is a CSF marker of neuronal damage during the development of Creutzfeldt-Jakob disease. Increased 14-3-3β protein is also found in CSF from patients with a variety of neurological disorders. The goal of this study is to determine whether the levels of serum/CSF14-3-3β protein in patients with eosinophilic meningitis correlates with other CSF parameters and the patients clinical course. An in-house 14-3-3β ELISA was established to determine the dynamic changes of 14-3-3β expressions in mice and patients infected with *Angiostrongylus cantonensis.* In a cohort study among nine Thai laborers with eosinophilic meningitis from eating raw snails, we examined the CSF weekly while patients were hospitalized and followed up the serum for 6 months. Forty BALB/c mice were randomly allocated to five groups: control, D7, D14, D21, and D21+dex (10ug dexamethasone was given daily via intra-peritoneal route from D7 to D21). The mice in the infection groups were given 50 *A. cantonensis* infective larvae by oral inoculation on day zero and sacrificed on days 7, 14, and 21 post-infection (PI). In each group, serum and CSF were obtained for 14-3-3β concentrations measurement by in house ELISA. All of the nine patients with eosinophilic meningitis underwent a total of 23 lumbar punctures. The elevated 14-3-3β level was detected in the CSF of eight out of nine (81%) patients during initial hospitalization. After 2 weeks of treatment, all patients showed a declined level or clearance of 14-3-3β protein in the CSF. By developing an in-house ELISA for measurement of 14-3-3β protein, it was found that the serum 14-3-3β level was significantly increased in patients during initial visit. After treatment, the serum 14-3-3β level in meningitis patients rapidly returned to normal levels. There was a trend of correlation between initial CSF 14-3-3β level with pleocytosis or eosinophilia in the CSF of patients with eosinophilic meningitis (Spearman's correlation test, r = 0.6, *P = 0.089)*. In the mice infected with *A. cantonensis*, we identified that there was a significant increase in the CSF levels of 14-3-3β 3 weeks after infection (p=0.027) and steroid treatment could reduce the expression of 14-3-3β (p=0.023). The serum 14-3-3β levels were not changed with the infection. In conclusion,14-3-3β concentrations may constitute a useful marker for disease severity and follow up in patients and mice with eosinophilic meningitis caused by *A.cantonensis*.

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TRAVMIL: TRAVEL-RELATED INFECTIOUS DISEASE RISK ASSESSMENT, OUTCOMES AND PREVENTION STRATEGIES AMONG DEPARTMENT OF DEFENSE BENEFICIARIES

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Large prospective studies describing risk factors for and location specific frequencies of infectious diseases in travelers are needed to improve guidelines for preventive advice, vaccinations, self-treatment strategies and prophylactic medications. The TRAVMIL study applies a novel approach to prospectively describe the epidemiology of travel related infectious diseases in US Department of Defense personnel and their beneficiaries and evaluates compliance rates and effectiveness of risk reduction and self treatment strategies. The study focuses on traveler's diarrhea (TD), febrile illness, and influenza like illness (ILI), combining clinical and laboratory data. Adults and children seen at two naval hospital travel clinics have been enrolled since 2010. Clinical information is obtained by medical record extraction, pre- and post travel surveys and illness diaries completed during travel. Laboratory specimens obtained include paired pre- and post-travel blood samples for serologic and immunologic testing, self-collected stool samples smeared on Whatman FTA® cards during travel from participants with and without TD, self-collected finger stick blood samples transferred to Whatman FTA® cards from those who develop a febrile illness during travel and oropharyngeal swabs from those who develop an ILI during travel. Serologic testing for pathogens associated with TD and febrile illness is performed on pre- and post-travel serum samples. Polymerase Chain Reaction (PCR) assays for viral pathogens are performed on oropharyngeal swabs. PCR is employed to detect *Plasmodium* sp., Dengue virus, *Leptospira* sp., chikungunya, and *Rickettsia* from blood blots. A multiplex PCR test to detect common diarrheal pathogens (*Salmonella*, *Shigella*, *Campylobacter*, Enterotoxigenic *E. coli,* Enteroaggregative *E. coli*, *Giardia*, *Cyclospora*, *Cryptosporidium*, Norovirus) in stool smears is under development. Preliminary data regarding assay development and frequency of infectious diseases among travelers will be presented.

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ANTIMALARIAL PRESCRIBING PRACTICES AMONG INFECTIOUS DISEASES PHYSICIANS AT A SINGLE DOD TRAVELER'S HEALTH CLINIC

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Factors associated with antimalarial prescribing practices among physicians are not well described. We performed a retrospective chart review to examine antimalarial prescribing practices among four infectious disease physicians at a military traveler's health clinic between April 2007 and October 2009. A total of 1,052 travel clinic visits were evaluated. Adults aged 18-65 accounted for 65% of travelers. Twelve percent of patients were < 12 years old. The most common regional destinations were Africa (29%), Southeast Asia/Pacific (21%), Central America/Caribbean (20%), Central Asia (11%) and South America (8%). Six hundred and sixty two (59%) travelers were given antimalarial prescriptions, the majority for atovaquone/proguanil (50%) followed by mefloquine (28%) and off-label primaquine primary prophylaxis (15%). Chloroquine (4%) and doxycycline (3%) were rarely used. Atovaquone/proguanil was the most often prescribed for all regional destinations and was prescribed frequently for travelers to Central America/Caribbean (17%). Duration of travel did not influence whether chloroquine or atovaquone/proguanil was used in chloroquine sensitive areas. However, in chloroquine resistant regions, duration of travel was associated with antimalarial choice. The mean duration of travel was 3.6 weeks for those prescribed atovaquone/proguanil and 7.6 weeks for mefloquine. The mean duration difference (4 weeks) was significant (95% CI = 1.3 - 6.7). Physician 1 was more likely to prescribe atovaquone/proguanil than all other physicians combined (OR 2.65, 95% CI 1.87 - 3.77, p <.0001). Physician 2 was more likely to prescribe off-label primaquine for primary prophylaxis than all other physicians combined (OR 7.49, 95% CI 4.08 - 13.74, p <.0001) primarily for *P. vivax* predominant areas. This study suggests that factors such as duration of travel, chloroquine resistance, prevalence of *P. vivax*, and individual prescriber preferences account for differences in antimalarial prescribing practices. Additional data, including prescriber resource utilization will be presented.

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ARTEMETHER, DIHYDROARTEMISININ AND LUMEFANTRINE DO NOT INDUCE *IN VITRO* DRUG METABOLIZING ENZYMES AND METABOLISM OF ORAL CONTRACEPTIVES

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The goal of this study was to evaluate *in vitro* the components of Coartem/Riamet (artemether and lumefantrine) and the active metabolite dihydroartemisinin (DHA) for their potential to induce drug-metabolizing CYP enzymes and the metabolism of oral contraceptives. The experiments were conducted according to the FDA drug drug interaction guidance. The assessment was done *in vitro* in cryopreserved primary human hepatocytes of at least three individual donors. Induction of mRNA, relative to the vehicle control, was determined by real-time PCR and evaluation of changes in cytochrome P450 (CYP) enzyme activities were assessed after 48-h induction periods by LC/MS/MS analysis of CYP-selective probe substrate metabolism. Metabolism of the oral contraceptives was tested by HPLC analysis. Human hepatocytes were incubated with the three test substances up to concentrations which exceeded their therapeutic concentrations by a factor of 10. Ethinyl estradiol and levonorgestrel were selected as active ingredients of oral contraceptives and were tested at their therapeutic concentrations of 1 nM and 20 nM, respectively. Rifampicin at 0.1, 1, and 20 µM, and phenobarbital at 1000 µM were used as positive controls for induction of genes regulated by PXR and/or CAR like CYP2B6, CYP2C, and CYP3A; β-napthoflavone at 10 µM was included as positive control for AhR-mediated induction of genes like CYP1A. Artemether, DHA, and lumefantrine were determined not to be inducers of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, or CYP3A enzyme activity in hepatocytes or CYP1A1, CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP3A4, or CYP3A5 mRNA. Metabolism of ethinyl estradiol and levonorgestrel was determined not to be induced by artemether, DHA, and lumefantrine. As per FDA criteria, these conclusions were based upon the levels of mRNA or activity at least less than 2-fold, with respect to the vehicle control, and/or less than 40% of the maximal positive control induction response, indicative of a non-inducer *in vitro*.

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ASSESSMENT OF THE THERAPEUTIC EFFICACY AND TOLERABILITY OF THE ARTESUNATE/AMODIAQUINE COMBINATION AND ARTEMETHER/LUMEFANTRINE COMBINATION, FOR THE TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN THE DEPARTMENT OF CHOCÓ (COLOMBIA)

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Malaria due to *Plasmodium falciparum* is a public health problem in more than 100 municipalities of Colombia. The country is using artemether + lumefantrine (AL) fixed-dose combination for uncomplicated *P. falciparum* malaria. One alternative treatment is the WHO prequalified fixed-dose combination of artesunate and amodiaquine (ASAQ), which can be given in a simpler dosing regimen. This open controlled clinical trial, comparing AL and ASAQ efficacy and safety profiles was carried out from August 2008 to September 2009, in Chocó, a highly endemic area for *P. falciparum* malaria,. Adult patients diagnosed with uncomplicated malaria (n=210) were randomized into two arms, one receiving ASAQ (n=105) and the other AL (n=105). Clinical and parasitological parameters were monitored over a 28-day follow-up period to evaluate drug efficacy and safety. There were no losses to follow up. The mean age of the enrolled patients was 37.5 years without differences between study arms. Both therapies were similarly well tolerated, with the exception of epigastric burning sensation, which occurred in 1 patient during ASAQ treatment and 14 patients during AL treatment (p<0.001). D28 efficacy of ASAQ was 100%, and that of AL was 99% (NS). On average, blood smears became negative after one day of ASAQ treatment and after two days of AL treatment; gametocytes disappeared after 2 days of treatment in the ASAQ arm compared to 4 days in the AL arm. In this study, the efficacy and safety profile of the ASAQ combination was similar to that of AL. These findings support the use of ASAQ as an alternative treatment for uncomplicated *P. falciparum* malaria in Colombia.

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SOLUTION FOR THE POWER SUPPLY OF PORTABLE ULTRASOUND MACHINES IN THE DEVELOPING WORLD IN AREAS WITHOUT ELECTRICITY

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Portable ultrasound has been shown to be an important adjunct to clinical diagnosis and patient management in resource limited areas in the developing world. There have been several impediments to its further expansion in remote settings. One impediment has been the lack of a reliable and stable electrical source to power the machines or to recharge the batteries. Formerly scanning has been limited to one battery discharge- 45 minutes to 1.5 hours, dependant on the machine and

ambient temperature. We demonstrated the reliability and effectiveness of a commercially available solar charging unit to provide power for 3 different ultrasound machines in areas lacking electricity. The study was a retrospective observational study of four different locations. Locations included Mt. Everest, Bangladesh, Haiti, and Mali. Ultrasound machines used for the study included a SonoSite Nanomaxx and Micromaxx, and GE Logiqbook. The power source evaluated was a Brunton brand 26 Watt folding solar panel, lithium ion polymer battery and power inverter. Scans were preformed from 2 to 8 hours per day with no down time experienced secondary to electrical failure or mechanical dysfunction. Environmental extremes included ambient temperatures from -10 degrees to 113 degrees Fahrenheit. The combination of the Brunton battery, solar panel and inverter is a reliable, commercially available solution for powering portable ultrasound machines in developing areas lacking electricity.

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HYPERTENSION IN RURAL CENTRAL INDIA: A STUDY OF PREVALENCE AND POTENTIAL RISK FACTORS

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The villages in the area surrounding Jamkhed, India have undergone a dramatic epidemiological transition with a shift from communicable disease to more chronic non-communicable conditions in the past two decades and due to the work of the Comprehensive Rural Health Project, a non-governmental organization operating in the area since 1970. To develop a sound prevention and management strategy we collected data on the prevalence of hypertension and its potential risk factors. In summer 2010, we randomly surveyed households in six villages surrounding Jamkhed, a township of 40,000 in rural central India. Using an oral questionnaire, we evaluated 226 subjects above the age of 40 for risk factors for hypertension. We measured blood pressure in both arms and the abdominal girth. Mean age was 56 years old (40-85). 80% were farmers and 56% female. 30% met criteria for high blood pressure (as defined by systolic BP greater than/equal to 140 mm Hg or diastolic BP greater than/equal to 90 mm Hg) with higher rate in men. 6% were at risk of Hypertension. Increased abdominal girdle was associated with high blood pressure. Diet was carbohydrate-based with high salt intake. The most common risk factors for hypertension were tobacco use, increased abdominal girth, increased age, and family history. Prevalence of high blood pressure in this rural area with subsistence farming is alarming and warrants further investigation. This study helps to raise awareness for the public and healthcare providers about hypertension. Strategies to prevent and manage hypertension should be considered.

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PRE-TRAVEL PREPARATION OF U.S. TRAVELERS PROVIDING HUMANITARIAN SERVICE, GLOBAL TRAVEPINET, 2009-2010

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Humanitarian service workers (HSW) face risks different from those faced by persons traveling for other purposes. HSW are at higher risk due to close contact with local communities in countries high-risk for disease. We describe characteristics of HSW visiting clinics pre-deployment from January 2009_January 2011 in Global TravEpiNet (GTEN), a national network of clinics providing care to International travelers. HSW were categorized as those who traveled for work involving missionary, nonmedical, medical service or a combination therein. Vaccination specifications and holoendemnicity were defined according to CDC recommendations. We also performed a subanalysis involving HSW traveling to Haiti in 2010. Fifteen percent (1946/13235) of GTEN travelers indicated they were HSW; 59% of these were aged 18_35 years, and 64% were female. HSW were stratified as missionary (26%), nonmedical (39%), and medical service workers (26%); 9% reported >1 HSW category. The most common destinations were Haiti (15%), Uganda (6%), Tanzania (5%), Kenya (5%), and Ghana (5%). Of HSW going to countries holoendemic for malaria, 95% received antimalarial chemoprophylaxis. Of HSW going to countries holoendemic for yellow fever, 96% of HSW were already immune or received yellow fever vaccine at the clinic visit. However, 20% of medical workers either did not have self-reported pre-existing varicella immunity or did not receive varicella vaccine. Further, 25% of missionaries either did not have self-reported pre-existing Hepatitis B immunity or did not receive vaccine. The number of HSW deploying to Haiti increased markedly following the 2010 earthquake; the median duration of stay in Haiti for 2010 HSW was 8 days. In summary, the majority of GTEN HSW are under 35, more likely female, and often travel for short durations. Some HSW may not be fully immune to illnesses common in destination countries. Travel medicine clinicians should utilize the pre-travel consultation as a platform to immunize for routine and travel-related vaccinations, especially when the traveler might be at high risk.

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EPIDEMIOLOGY OF *CANDIDA* AMONG WOMEN VAGINAL TRACT IN QATAR

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Vaginal symptoms are a leading cause for women patients to visit her gynecologist. Since the incidence of candidiasis has increased dramatically during the last decade, thus risk factors and symptoms must be considered in order to deal with this disease and avoid its complication. The aim of this study was to determine the frequency and distribution of *Candida* spp. in women with vulvovaginal symptoms. High vaginal swabs were collected from women patients refer to Women Hospital in Doha, Qatar in summer 2010 suffering from abnormal vaginal discharge, leaking, itching. Samples were identified using culture method and Vitiek II system for species conformation to record the species types. For each patient, age, nationalities, pregnancy, vaginal discharge, leaking were recorded as risk factors for vulvovaginal candididas. During the period of the current study (June-November 2010), a total number of 222 women were found to be infected with at least one of the following *Candida* species: the most predominant species was *C. albicans* (77.5%) followed by *C. parapsilosis* (13.5%), *C. tropicalis* (7.2%), with less detected species for *C. kruzie* and *C. glabrata* (2.7%). However these results indicate that factors associated with age and pregnancy may influence the occurrence of *Candida* spp. in women with vulvovaginal symptoms. The frequency distribution of *C. albicans* in pregnant women was (30.6%) and (32.4%) with women complains from vaginal leaking. The incidence of vulvovaginal candidiasis was found to be 57.65% in women less than or equal to 30 years old compared with 42.34%in women greater than 30 years of age. With relation to patients nationalities, (52.52%) of non- Qatari women were suffering from candidal vaginitis compared to (46.74%)of Qatari women. In conclusion, the limitations of the present study, it has not documented the history of recurrent infection and antimicrobial use. The current clinical data listed in patients were limited. As a result, sufficient data were unavailable to evaluate the risk of contraceptive practices and antimicrobial use of vaginal candidiasis.

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SEVERE DENGUE VIRUS INFECTION IN PEDIATRIC TRAVELERS VISITING FRIENDS AND RELATIVES IN THE BRONX, NEW YORK

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Dengue fever (DF) has been recognized to be the most frequent cause of a systemic febrile illness in travelers returning from tropical regions other than Africa. Hitherto travel-associated severe dengue infections have been mostly described in adult international travelers. The objective of this report is to analyze the travel, clinical and laboratory characteristics of children who were diagnosed with DF after return from international travel. Data was abstracted from charts of pediatric patients who were diagnosed with DF at the Bronx-Lebanon Hospital Center betweeen May 2007 and December 2010. A commercial dengue virus IgM capture ELISA and a dengue virus IgG indirect ELISA was used for diagnostic testing. An IgM/IgG index ratio ≤1.07 was applied to distinguish secondary from primary dengue virus infection. The WHO criteria were used to diagnose dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). We identified 8 children with acute dengue virus infection (3 children (38%) with severe dengue infection [DHF, n=2] and [DSS, n=1]). All had traveled to visit friends or relatives (VFR) for the median duration (range) of 32 days (10 days -4.3 years) in the Dominican Republic (88%) or Puerto Rico (12%), and presented ill after the median time since return (range) of 6 days (1-11), (63% females, 75% U.S. born, median age [range] of 13.6 [0.3-17.6 years). All presented with an acute febrile illness accompanied by gastrointestinal complaints (63%), myalgia (50%), petechial rash (38%), dehydration (25%), and headache (13%). Relevant laboratory findings included leukopenia (63%), thrombocytopenia (75%), elevated serum alanine aminotransferase (38%), low serum albumin (38%), and elevated hematocrit (25%). Sonogram revealed ascites (50%), pleural effusion (38%), gallbladder thickening (38%), and heterogenous liver parenchyma (25%). Of the children with severe dengue virus infection, 2 teenagers (DHF) had a secondary immune response, and one infant (DSS) had a primary immune response. Due to increasing global migration a growing proportion of children traveling to tropical and subtropical regions are at risk of exposure to dengue virus infection. Therefore children of immigrant families originally from dengue-endemic countries may benefit from competent pre-travel advice, and may represent candidates for a future dengue vaccine.

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RECOMBINANT SINDBIS/VENEZUELAN EQUINE ENCEPHALITIS VIRUS FOR THE DETECTION OF SEROTYPE-SPECIFIC IMMUNOGLOBULIN M ANTIBODIES IN MEXICO

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Venezuelan equine encephalitis virus (VEEV) is an important arboviral pathogen in Central and South America and has caused numerous outbreaks in both equids and humans since the 1920s. Subtype I VEEVs comprise 5 varieties, including subtypes IAB, IC, ID, IE and IF, four of which are classified as HHS select agents. To avoid the risk and regulatory difficulties of working in high containment under select agent regulations, vaccine strain TC-83, which belongs to the IAB variety, is commonly used as an antigen for detecting immunoglobulin M (IgM) antibodies in response to all subtype I VEEV strains. However, it is less sensitive for detecting IgM antibodies induced by other VEEV varieties, and therefore, it limits the accuracy of etiologic diagnostics. To explore the potential application of a recombinant Sindbis virus (SIN)/VEEV for clinical diagnosis, we compared the sensitivity of SIN/VEEV that was derived from subtype IE VEEV (SIN/VEEV-IE) to the vaccine strain TC-83 for IgM detection in sera from either naturally exposed humans in Mexico or experimentally infected equids. When using goat anti-human IgM to test human VEEV-IE positive sera in an IgM capture ELISA, we observed a higher sensitivity of detection when using SIN/VEEV-IE (80%) than TC-83 (60%). When VEEV-IE-specific monoclonal antibody 1A1B-9 was used to in a sandwich ELISA, SIN/VEEV-IE generated 100% positivity in both human and equine sera, whereas TC-83 failed to detect positive sera, confirming the enhanced antigenic accuracy with recombinant virus. Endpoint IgM antibody titers using SIN/VEEV-IE were only slightly lower (2-fold) when compared to a wild-type VEEV IE strain. Our results indicate that recombinant SIN/VEEV-IE provides a better target than TC-83 for IgM detection of a recent infection by subtype IE VEEV, potentially replacing the use of wild-type VEEV in serological tests.

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FACTORS ASSOCIATED WITH APPROPRIATE HOUSEHOLD DIARRHEA CASE MANAGEMENT IN HOSPITALIZED CHILDREN, NYANZA PROVINCE, KENYA, 2007

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Diarrhea is a major cause of morbidity and mortality in children under five in Nyanza Province, Kenya. Oral rehydration therapy (ORT), defined here as initiating or increasing oral rehydration solution (ORS), breast milk, watery porridge, homemade sugar-salt solution, and/or other fluids, is an affordable and effective treatment for childhood diarrhea. The goal of this study was to identify modifiable factors associated with ORT use in children with diarrhea requiring hospitalization. In 2007, we conducted a cross-sectional study of caregivers of children under five who were hospitalized for diarrhea at two district hospitals in Nyanza Province. We developed a behavioral model that included constructs hypothesized to be associated with ORT use, including perceived positive and negative attributes of ORT, self-efficacy, beliefs regarding diarrhea and treatment, and demographic factors. Using logistic regression, we identified factors associated with ORT use *within* each behavioral construct. We tested those variables associated with ORT use within each construct in a final cross-construct logistic regression model. The median age of the 119 respondents was 21 years (range 15-40 years) and median age of the children was 10 months (range 1 month to 3 years). Twenty one (18%) respondents had less than primary school education, 73 (61%) completed primary school, and 26 (22%) completed some secondary school. ORT use was reported by 93 (78%) respondents, with 73 (61%) reporting use of ORS specifically. The following factors were independently and significantly associated with ORT use in the final cross-construct mode: a caregiver believing a child could die (OR_{adj} 4.2 95% CI 1.4-12.8, p = 0.01), a caregiver feeling she knows how to prepare ORS (OR_{adj} 3.4, 95% CI 1.2-9.8, p = 0.03), and advisors to the caregiver recommending ORS and/ or increased fluids (OR_{adj} 3.8 95% CI 1.3-11.1, p = 0.01). ORT use was relatively common among children requiring hospitalization for diarrhea in rural western Kenya. Our results suggest that risk perception, self efficacy, and social support are important determinants of ORT use. Interventions to improve diarrhea case management should educate caregivers to appreciate the risk of dehydration from diarrhea, emphasize ORT's ability to prevent and treat dehydration, and enhance self efficacy of caregivers by encouraging them to initiate ORT at home based on its ease of use, affordability, and effectiveness.

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DYING WITH THEIR BOOTS OFF: NON-TRAUMATIC DEATHS AMONG AMERICAN TROOPS IN VIETNAM, 1960-1975

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The medical literature, ranging from ancient to modern sources, describes a staggering number of causes of combat-related mortality and morbidity. Moreover, the extent of such casualties broadened with the development of new modes of military technology. Traumatic injuries due to lead shot, rifle bullets, and high explosives undoubtedly lead the list among the aetiology of these events. Such categorization of causes of wartime deaths, however, has expanded even more significantly in the post-WWII era. The Southeast Asia Combat Area Casualty File, for example, contains a rich array of mortality data concerning American troops who died between 1960 and 1975 in Vietnam. The purpose of this study is to analyze cases in this dataset that died as a result of non-traumatic injuries (*e.g.,* heart attack, suicide, etc.), aetiologies more often associated with civilian rather than military life. Among the twenty-one official death categories listed in the Southeastern Asian Combat Casualty File about half of them relate directly to traditional military operations, *e.g.*, gunshot wound, grenade shrapnel, etc. The remainder, however, include deaths from suicide, heart attack, "misadventure" etc, the epidemiology of which will comprise the focus of this study.

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INTESTINAL BACTERIAL/VIRAL AND PARASITE CO-INFECTION IN INFANTS WITH ACUTE DIARRHEA

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The estimated worldwide death rate from diarrheal diseases is about 2.2 million deaths per year as reposted by the WHO. Diarrheal infections may be caused by an array of bacterial, viral, or parasitic pathogens. Some cases have 1 single defined cause, others do not have any defined cause, and a substantial number (one third) are caused by multiple pathogens. It's very little known about confection of bacteria and parasite and how it reflects on clinical course of acute diarrhea, especially in infants. Totally data of 168 children cases with acute diarrhea were analyzed. The data collected included census data, history of disease (onset, severity, duration of disease), common blood count, stool bacteriological (culture for intestinal bacterial pathogens following world wide accepted technique), O&P (ova and parasites microscopy) and ELISA for rotavirus antigen (IDEIA, Dako Ltd., United Kingdom) examination. The data was analyzed with "R" software (http://www.R-project.org/). The average age of children was 13 months (±8,2 months) *Salmonella typhimurium* was isolated in 14 patients (8.34%). Rotavirus was detected in 47 patients (27.97%). The parasites detected in the following sequence: 26 Blastocyst (15.48%), 25 Pinworms (Enterobius vermicularis) (14.88), 3 Pinworms (*Enterobius vermicularis*) + Blastocyst (1.79%), 1 Pinworms (*Enterobius vermicularis*) + *Giardia intestinalis* (0.6%), 15 *G. intestinalis* (8.93%), 2 *G. intestinalis* + Blastocyst (1.19%) and no parasite detected in 96 patients (57.14%). There was significant difference in duration of hospitalization in children with bacterial and/or rotavirus diarrhea and children with co-infection of bacteria/virus and parasite ($p<0.001$). The parasites like Blastocyst, pinworms and *G. intestinalis* are very common in children with diarrhea. Due to poor control of antimicrobial utilization the bacterial pathogens is rarely isolated. Though rotavirus is the predominant viral diarrheal agent among children the intestinal parasites burden like *G. intestinalis* is underestimated and must be considered. In general the children with bacteria/virus and parasite co-infection have longer staying in hospital comparing the ones with bacterial and/or viral diarrhea. We recommend performing O&A stool examination in all children with acute diarrhea.

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LABORATORY DIAGNOSTIC DETERMINATION OF C-REACTIVE PROTEIN IN AIDS POPULATION IN ETHIOPIA

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The aim of the study was assess of CRP testing as an important indicator of bacterial infection in the diagnostic of inflammation and monitoring the effectiveness of antibiotic treatment in terms of Ethiopia. In admission we were willing to extend the basic laboratory in Kibre Mengist with assessment of clinical outcome and correlation with test results provided the diagnosis only on the basis of clinical signs of disease. The diagnostic of hospitalized patients and outpatients in a hospital in Kibre Mengist based on clinical signs of disease, using rapid diagnostics tests (RDT) and quantitative determination of the value of CRP marker. The data were statistical analyses were carried out using SPSS software. The results clearly show that it is important to first obtain the consent to use RDT for screening in developing countries and accelerate reliable diagnostic and the treatment itself. The introduction of CRP examination is a clear contribution, especially in case of patients with ambiguous clinical picture of disease and especially where it is not possible to wait for the typical clinical signs, that means in case of young children and HIV/AIDS patients. Although the clinical diagnostic is cheap, wrong diagnosis leads to unnecessary prescriptions, which in turn supports the growth of resistance to drugs in the world and increases the cost of a new, effective treatment.

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ACUTE UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN INFANTS <5 KG BODY WEIGHT IN FOUR SUB-SAHARAN AFRICAN COUNTRIES - A DESCRIPTIVE STUDY

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Artemisinin-based combination therapy (ACT) is recommended as first-line treatment for infants ≥5 kg of body weight (BW) with uncomplicated *falciparum* malaria, but no ACTs are indicated in the population <5 kg. Published reports on malaria in younger infants are scanty, leaving a significant knowledge gap about the pattern and outcome of malaria in this sub-population. Hospital charts from 4 countries from Sub-Saharan Africa (Bénin, Democratic Republic of Congo, Nigeria, and Togo) were retrospectively reviewed for the period between 2006-2010 for inpatient neonates and infants <5 kg BW with a confirmed diagnosis of uncomplicated *Plasmodium falciparum* malaria. Clinical features, age group, treatment, and outcome were collected. The annual incidence ranged from <20 to >90 cases across hospitals and calendar years. The proportion of cases varied by age (≤28 days vs. >28 days): the proportion of infants in the older group was generally higher, but the younger group represented from <2% at one hospital in the Democratic Republic of Congo to >70% at another in Togo. The most frequent clinical presentation was fever, followed by dyspnea, crying, or vomiting. Whenever results were available, parasite load was generally low; <10%

of the infants presented with parasitemia >5000/µL. The majority of the infants were treated with oral quinine, except at two hospitals in Bénin and Togo, where AL and intramuscular artemether were administered, respectively. Although infrequent, malaria in neonates and infants <5 kg of BW does exist in certain endemic countries and calls for appropriate treatment. Further clinical evidence regarding the use of ACTs in this population is warranted.

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MATURATION OF DENGUE VIRUS NONSTRUCTURAL PROTEIN 4B IN MONOCYTES ENHANCES PRODUCTION OF DENGUE HEMORRHAGIC FEVER-ASSOCIATED CHEMOKINES AND CYTOKINES

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Of the 50-100 million dengue virus (DENV) infections worldwide each year, approximately half-million hospitalizations occur. Mechanisms explaining why some individuals progress to severe disease are unclear. However, DENV preferentially infects peripheral blood monocytes, which secrete elevated levels of chemokines and cytokines in patients progressing to severe disease. Of the ten DENV proteins, several nonstructural proteins (NS) including NS4B and NS5 are capable of inhibiting interferon signaling. For the first time, we report that NS4B and NS5 expressed in monocytes are potent inducers of immunomediators associated with severe disease. Further, we demonstrate that cleavage of the NS4AB polyprotein by the viral protease NS2B3(pro), via the intermediate 2KNS4B, is significantly more potent than NS4B or NS5 alone, inducing immunomediators to levels similar to DENV infection. The 2K-signal peptide is not required for the induction of immunomediators yet it plays a synergistic role with NS4B. These data suggest that maturation of NS4B is primarily responsible for the induction of immunomediators associated with severe disease. Given that NS4B structures are conserved across flaviviruses, NS4B may be an attractive target for the development of Flavivirus-wide therapeutic interventions.

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RISKS FACTORS AND INCIDENCE OF DENGUE IN A PROSPECTIVE COHORT STUDY IN MARACAY, VENEZUELA

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Dengue has become the most important vector-borne viral disease in the Americas. Dengue in Maracay, Venezuela, is hyperendemic with co-circulation of the 4 viral serotypes. The increment of dengue transmission in Venezuela has coincided with an increase in the incidence of severe disease which in 2010 reached nearly 10% of all cases. A cohort study of 2000 individuals aged 5-30 years was established between August and December 2010 in Maracay city. Baseline epidemiological data and blood samples were obtained. Annual cross-sectional sampling will take place. Febrile cases are identified through passive and active house-to-house surveillance. Blood samples will be analysed using hemagglutination inhibition test and plaque reduction neutralization test to determine the incidence of symptomatic and inapparent dengue infection and the possible association with primary or secondary infection. Epidemiological data will be used to identify potential risk factors for dengue infection. Preliminary results will be presented and discussed.

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VISUALIZATION OF DENGUE RNA REPLICATION IN LIVING CELLS

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Dengue virus (DENV) is an enveloped virus with a single-stranded, 10.7 kb positive-sense RNA genome. There are two complementary sequences in the 5′ and 3′ untranslated regions that interact with each other to circularize the viral genome. Following circularization, the viral RNA-dependent RNA polymerase, known as NS5, plays a crucial role in the initiation and regulation of RNA synthesis. However, it still remains controversial whether the DENV genome exists primarily as single or double stranded RNAs in host cells. Because of the lack of a direct method to investigate each form of RNA in living cells, localization of the viral RNA genome has also to be elucidated. Here, we introduced two fluorophore specific RNA aptamers; Malachite green RNA aptamer and Hoechst RNA aptamer to directly visualize the viral RNA replication process and differentiate each strand of RNA *in vivo*. Both RNA aptamers were constructed into a new reporter system for investigate the mechanism of DENV RNA replication, transportation and localization process in human cell lines in real-time. We will further discuss the use of the system to trace the life time of non-coding RNAs in living cells.

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VECTOR CONTROL AND SURVEILLANCE DURING A MAJOR OUTBREAK OF DENGUE IN A COASTAL RED SEA AREA: PORT SUDAN CITY

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An unprecedented dengue outbreak occurred on the coastal Red Sea in Port Sudan during the period (27 February - 25 June)2010. A vector control response plan to the outbreak had mainly entailed house inspection and insecticide space spraying against the dengue vector, as well as integration of vector surveillance work to evaluate the response plan. A total of 3223 enrolled dengue cases in Port Sudan was reported over 17 epidemiological weeks. A total of 3048 houses were inspected during the vector surveillance work resulting in collection of 19,794 larvae and 3,240 pupae of *Aedes aegypti*. A significant decrease in the entomological indices was shown during the observed period: House Index (HI) has declined from 100% to 16% ($F= 57.8$, $p<0.001$), while pupal/demographic (P/D) index has decreased from 0.77 to 0.10 ($F= 3.06$, $p<0.01$), on the 9th and 21st weeks, respectively. Accordingly, this decline has been accompanied by a decrease in the numbers of dengue cases from a peak observed on the 13th week (341 cases) to the lowest on the 25th week (49 cases) per week. Using regression line analysis, a significant relationship exists between the measured entomological parameters and the trend of dengue cases on the next weeks ($R2=0.83$; $F= 23.9$, $p<0.001$). This study clearly shows entomological surveillance is sensitive to evaluate vector control and monitor dengue epidemics in such coastal area. Integration of epidemiological and entomological indicators should be the basis of a surveillance system for the emerging dengue on the coastlines of Red sea.

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DUAL ROLE OF INTERFERON RESPONSE IN DENGUE INFECTION

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Over the past quarter century, risk for infection with one of four serotypes of dengue virus (DENV) has markedly increased. Currently, 40% of the world's population lives in endemic regions. Understanding the mechanisms of DENV emergence has been complicated by the lack of animal models of transmission, where susceptibility to infection establishment by non-adapted virus and natural routes of infection are critical. *In vivo* studies have also shown the importance of the type I interferon and JAK/STAT pathways, and specifically, the IRF family in dengue and flavivirus infection. We introduce a novel model for transmission, an IRF3/7 double knockout (DKO) mouse strain (C57BL/6 background) which has a delayed and significantly blunted systemic type I interferon response. We hypothesized that early type I interferon only, and not type II, is critical for the inhibition of dengue virus infection establishment, and thus successful transmission. We further evaluated this hypothesis via a natural transmission route to determine whether the relative importance of types I and II interferon is changed by exposure route. We were able to established 100% infection rate in these DKO mouse with three different, non-adapted strains of dengue virus of two serotypes. Type II interferon appears necessary for viral clearance, but is apparently not as critical for the initial establishment of dengue infection *in vivo*. We conclude, then, that type I interferon, and not type II, is the essential mediator of successful transmission of dengue virus.

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AEDES AEGYPTI IN CAPE VERDE: STATUS AND RESEARCH PERSPECTIVE

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Aedes aegypti the most competent dengue vector is present in the all islands of Cape Verde. In 2008 were the first dengue cases in the country and in September 2009, Cape Verde was ravaged by an epidemic of dengue. During this first outbreak of dengue in Cape Verde, were registered 25 071 suspected cases nationwide, 6 747 confirmed cases, 174 cases of Dengue hemorrhagic fever/Dengue shock syndrome and 4 deaths (MOH data)caused by serotype DEN-3, and occurred from September to November 2009. Several factors may be responsible for this epidemic, the rapid and disorganized urban growth and consequent negative impact on sanitation, a relatively long rainy season with rainfall and the multiplication of air links with regions of the world where presence dengue is circulating. Sensibility tests on *Ae. aegypti* with larval sampling of populations from five areas of Santiago Island and São Filipe, using the diagnostic dose of temephos recommended by WHO, showed mortality rate between 95 to 100%. With these experiences, we can confirm that the population of *Aedes* in Cape Verde is sensible to temephos. This could be explained by the low use of insecticides in vector control. So is necessary to have a constant surveillance of mosquitoes susceptibility against insecticides, as well their effectiveness in the field in order to avoid high level of resistance, that facility the management of resistance case if detected. Results of dissemination and transmission rates of *Ae. aegypti* orally exposed to DEN-3, Chikungunya (CHIK) and Yellow Fever (YF) viruses show that population of *Ae. aegypti* collected in Praia (Cape Verde) has high vectorial competence to CHIK and YF. In the case of introduction of those viruses in the country can be cause major epidemics cases such as occurred with dengue. So, with the increasing incidence of dengue, yellow fever and Chikungunya worldwide, especially in tropical countries, it is crucial to develop studies on ecology, mapping of insecticides susceptibility/resistance, vectorial competence and genetic populations of *Ae. aegypti* in Cape Verde Islands. For those studies, we will create partnerships between Local Institutions and International Agencies. All entomological data will be associated with epidemiological, social and environmental data in order to reinforce the national program activities and to develop some new approaches in vector control.

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THE SEROPREVALENCE OF DENGUE FEVER IN SOLOMON ISLANDS

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There is lacking in the prevalence data of dengue fever in Solomon Islands in recent years. During 2009 and 2010, we investigated the seroprevalence of dengue fever infections of school students in Solomon Islands. Under IRB approval, we conducted this survey and used the Panbio Dengue IgG Indirect ELISA to detect IgG antibodies to dengue antigen serotypes (1, 2, 3 and 4) in serum, as an aid in the clinical laboratory diagnosis of patients with clinical symptoms and past exposure consistent with dengue fever. We then used the PRNT (plaque-reduction neutralization test) of Japanese encephalitis (JE) to exclude the cross reaction of positive dengue specimens with Panbio Dengue IgG Indirect ELISA. At the same time, we examined the stool parasites for every enrolled subject using MIF (Merthiolate-iodine-formaldehyde) method. From the 588 serum specimens of study subjects under 20 years old, the seroprevalence rate of dengue is 62.2 % (366/588). The distribution of dengue in rural citizens (64.2%, 235/366) is significantly higher than that in urban citizens (35.8%, 131/366) ($p<0.001$). We analyzed the variables with multiple logistic regression method to find out the independent risk factors for seropositive dengue subjects, which indicated two important factors-- the rural citizens(OR 2.229, 95% CI 1.586-3.132), and the patients with hook worm infections(OR 1.686, 95% CI 1.095-2.596). The JE seropositive rate is 9.8% (38/386). On the contrary, the distribution of JE in urban citizens (17%) is significantly higher than that in rural citizens (5.2%)($p<0.001$). From this study, we did find the flaviviral infections existed in this community and dengue is prevalent in Solomon Islands which might be overlooked before. More intensive surveillance and control should be implemented.

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10 KDA INTERFERONΓ-INDUCED PROTEIN (IP-10) IS SPECIFICALLY ELEVATED IN DENGUE FEVER IN SUBJECTS WITH ACUTE FEBRILE SYNDROME AND PREDICTS THE DEVELOPMENT OF DENGUE HEMORRHAGIC FEVER: A CASE-CONTROL STUDY FROM COLOMBIA

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Causes of fever are often non-specific. Acute febrile syndromes like dengue fever, influenza and leptospirosis can have overlapping geographic distribution and clinical presentations but may require different treatments. This study was undertaken to identify biomarkers that can improve clinical discrimination of dengue fever, influenza and leptospirosis in individuals

with acute febrile syndrome in Bucaramanga, Colombia. Between 2003-2008, outpatients with an acute febrile syndrome were enrolled in a prospective cohort study. Serum levels of biomarkers from pathways of immune activation were measured in a random subset of subjects with dengue fever (n=113), leptospirosis (n=47), influenza (n=37) and healthy controls (n=14). 10 kDa interferonγ-induced protein (IP-10) was elevated in subjects with dengue fever compared to influenza, leptospirosis or healthy controls ($p<0.01$ for each). We evaluated the ability of IP-10 to discriminate between the three clinically similar syndromes by receiver operating characteristic curve (ROC) analysis. IP-10 was able to discriminate between dengue fever and leptospirosis or influenza with good diagnostic accuracy (area under the ROC curve (95% CI), p-value: 0.84 (0.77-0.89), $p<0.0001$ and 0.84 (0.77-0.90), $p<0.0001$ respectively). IP-10 levels at enrolment were elevated in individuals who developed dengue hemorrhagic fever (n=46, $p=0.014$). We used classification analysis (CRT) to integrate the clinical, laboratory and biomarker data into a single decision tree to discriminate between the three groups. A tree was generated where IP-10 was able to identify the majority of dengue cases and then cough, dizziness, and leukocyte count were able to further differentiate between dengue fever, leptospirosis and influenza. The model with IP-10 had a sensitivity of 80.4% and specificity of 94.6% to identify dengue fever compared to a sensitivity of 81.2% and specificity of 71.4% using clinical and laboratory parameters alone. In conclusion, IP-10 is a promising biomarker of dengue fever and may predict the development of dengue hemorrhagic fever.

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THE HISTORY OF DENGUE OUTBREAKS IN THE AMERICAS

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Dengue disease is caused by dengue virus serotypes 1-4 usually transmitted by *Aedes aegypti* mosquitoes. Currently, dengue is the most common endemo-epidemic viral arthropod-borne disease worldwide. We report the epidemic patterns of outbreaks in the Region of the Americas from 1600 to 2010. Dengue outbreaks reported in the literature and to the Pan American Health Organization (PAHO) were reviewed. Outbreaks were analyzed in 4 periods: A) Introduction of dengue in the Americas (1600-1940), B) Plan for the eradication of the *Ae, aegypti* (1947-1970), C) *Ae. aegypti r*e-infestation (1971-2000), D) Increased dispersion of *Ae. aegypti* and dengue virus circulation (2001-10). A) The first dengue epidemics occurred in 1635 in Martinique and Guadalupe. In 1818, an outbreak was reported in Peru (n~ 50,000 cases). In 1827 the first multi-country outbreak occurred (Virgin Islands, Cuba, Jamaica, Venezuela and many cities in the US). In 1912 a dengue epidemics was reported in Panama, Puerto Rico and northern Chile and Argentina. B) Successful control efforts resulted in a decrease in the number of outbreaks during 1948-1972 when the complete eradication of *Aedes* mosquitoes was certified in 21 countries. C) Outbreaks increased as the eradication program deteriorated. Approximately 702,000 dengue cases most DEN-1 were reported during 1977-80. In 1981 Cuba reported an outbreak with 344,203 cases, 10,312 cases of dengue hemorrhagic fever (DHF) and 158 deaths. Other epidemics occurred in Northern Brazil in 1982 and Rio de Janeiro in 1986, extending to Bolivia, Paraguay and Ecuador in 1988 and Peru in 1990. D) Outbreaks were reported in 2002 in Brazil [n=780,644; incidence/100,000 (I)= 452]; in 2007 in Paraguay (n=28,182; I= 500); in 2008 in Brazil (n=734,384; I=426); in 2009 in Bolivia (n=84,047; I= 864) and Argentina (n=26,612; I=71); and in 2010 in Honduras (n=66,814; I=1,016), Colombia (n=157,152; I= 685), and Brazil (n=1,004,392; I=525). An increasing number of outbreaks have been reported in the Americas during the last decades. Urgent global actions addressing integrated public policies for effective prevention and control are currently needed to avoid further spread of the disease.

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IDENTIFICATION AND CHARACTERIZATION OF NEUTRALIZING AND ENHANCING EPITOPES ON DENGUE VIRUS ENVELOPE PROTEIN

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Shotgun Mutagenesis technology was employed to identify detailed epitope maps for human MAbs derived from vaccine recipients and naturally-infected patients against the immunodominant envelope protein (prM/E) of Dengue virus (DENV). A comprehensive plasmid mutation library for DENV-3 prM/E was created in which every prM/E residue was individually mutated to a defined substitution, expressed in human cells, and analyzed for its effect on antibody reactivity and viral infectivity. For each MAb, we identified amino acids on prM/E that are required for antibody binding, and these residues were mapped onto the prM/E crystal structure to visualize epitopes. Our goals are to map and compare epitopes of antibodies against all 4 DENV prM/E serotypes and determine their role in viral protection and pathogenesis. The molecular and functional mechanisms by which MAb-epitope interactions contribute to the humoral immune response were characterized by measuring neutralization and antibody-dependent enhancement titers, MAb binding affinities, timing of inhibition (pre- or post-viral attachment), and the ability to support complement fixation. We expect that this approach will help define the range of immunodominant structures on prM/E and identify novel enhancing and neutralizing antibody epitopes that can be used for therapeutics, diagnostics, and vaccine development.

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DEVELOPMENT AND CHARACTERIZATION OF A STABLE REVERSE GENETIC SYSTEM OF A MALAYSIAN SYLVATIC DENGUE VIRUS TYPE 2 STRAIN (P8-1407)

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Dengue viruses (DENV) exist in two ecologically and evolutionarily distinct transmission cycles: 1) an urban cycle, which involves human reservoir and amplification hosts and peridomestic *Aedes* vectors, primarily *Aedes aegypti* and *Ae. albopictus* mosquitoes, and 2) a sylvatic (enzootic) cycle that involves transmission most likely among nonhuman primates and arboreal Aedes spp. Full-length infectious clones (FLIC) are powerful tools for the experimental investigation of the mechanisms that lead to viral emergence from the enzootic cycle. Here, we describe construction and characterization of the FLIC of the prototype Southeast Asian sylvatic DENV-2 strain P8-1407, isolated in 1970 from a Sentinel monkey in Malaysia. Viral cDNA was cloned under the control of a CMV promoter into the low-copy-number plasmid pACNR. To circumvent the inherent instability of the plasmid during its propagation in E. coli, an intron sequence encoding several stop-codons was introduced between the structural and non-structural protein genes of the viral genome. The resultant plasmid (pAC-P8-1407) was fully sequenced to verify its genetic integrity. Infectious virus was rescued by transfection of the pAC-P8-1407 FLIC DNA into Vero cells, where transfected cells yielded a productive virus infection as determined by an infectious center assay. Maximum titers of rescued virus were obtained 5 days post-transfection, which is consistent with the replication kinetics of the parental virus in Vero cells. Detailed genetic and phenotypic characterization of the rescued virus in cell culture and *Ae. albopictus* mosquitoes is currently ongoing. A subgenomic replicon of P8-1407 expressing the mKate2 fluorescent protein was also constructed and its application will be discussed.

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FAILURE OF HIGH TITER DENV-2 NEUTRALIZING ANTIBODIES TO PROTECT AGAINST SYMPTOMATIC AMERICAN/ASIAN DENV-2 INFECTION

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Following the introduction of a new lineage of American/Asian genotype of dengue virus serotype 2 (DENV-2), a large epidemic of dengue hemorrhagic fever (DHF) occurred in December 2010 in the Amazon basin city of Iquitos, Peru. While American genotype DENV-2 had been the cause of large outbreaks of dengue fever (DF) in the mid-1990s, little DENV-2 circulation had been detected in Iquitos since 2000. As part of on-going community-based longitudinal studies of DENV transmission in Iquitos, we used a plaque reduction neutralization test (PRNT) to determine the prevalence of serotype-specific neutralizing antibodies among residents of Iquitos immediately prior to the 2010 DHF epidemic. We found that 73.5% (95% confidence interval [CI]: 71.5%_75.4%) of the population had DENV-2 neutralizing antibodies, with a much higher prevalence among individuals 15 years of age or older (81.6%; 95% CI: 79.9%_83.3%) than children under the age of 15 (21.5%; 95% CI: 17.0% -- 25.0%). Among participants born after 2000, DENV-2 antibody prevalence was 4.8% (95% CI: 2.0%_7.6%). We found that many of the individuals who experienced symptomatic DENV-2 infection, as identified through door-to-door community-based surveillance, had robust DENV-2-specific antibody titers prior to infection, at a proportion similar to the general population. Greater than 50% of symptomatic infections occurred among individuals older than 15 years, despite the high prevalence of DENV-2 antibodies. These data suggest that antibodies generated against American DENV-2 strains do not provide robust protection against American/Asian strains. We plan to address this hypothesis by challenging pre-infection sera with American/Asian DENV-2 strains collected during the 2010 epidemic. Alternative hypotheses, including cross-reactive antibodies that do not accurately reflect past infection history, will also be explored. These studies have significant implications for understanding the immune response to DENV serotypes and for dengue vaccine development.

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INVESTIGATING THE HUMAN ANTIBODY RESPONSE TO PRIMARY DENGUE INFECTIONS

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Dengue viruses (DENV) are considered a major global health concern, infecting over 50 million individuals across the globe each year. DENV are mosquito-borne flaviviruses, existing as four serotypes (named DENV1 through 4). Following a primary DENV infection, individuals produce a complex mixture of serotype-specific and cross-reactive antibodies. This pre-existing immunity is thought to be sufficient to protect against re-infection with the same serotype, but may enhance infection and increase disease severity during a second infection with one of the other three DENV serotypes. Due to the complexity of the human humoral immune response, the binding, neutralization and enhancing properties of human polyclonal sera against DENV are not well understood. The goal of the current study was to fractionate antibodies in DENV-immune human sera using viral antigen or recombinant protein, and to investigate the role of specific antibody populations in DENV neutralization or enhancement. Depleted sera were tested for the ability to neutralize or enhance DENV in cell culture and in the AG129 mouse model of infection and disease. Both cell culture and animal studies demonstrated that after a primary infection, humans produce two distinct subpopulations of antibodies that are 1) type-specific and strongly neutralizing, and 2) cross-reactive, weakly neutralizing and enhancing. Further efforts to characterize neutralizing epitopes using recombinant envelope (rE) protein demonstrated that strongly neutralizing type-specific antibodies bound whole virus, but not the rE protein. We hypothesize that after a natural primary infection, neutralizing antibodies recognize epitopes that are only preserved on the whole virus, and not on rE dimers.

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VALIDATION OF THE RAPID DIAGNOSTIC TEST FOR DENGUE FEVER/DENGUE HEMORRHAGIC FEVER WHICH DETECTING EITHER ANTIGEN OR ANTIBODY

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Dengue fever (DF) is endemic in Thailand and dengue hemorrhagic fever (DHF) has been reported in 2010 with double increase to 2009. The objective of this study was to evaluate the rapid diagnostic test for Dengue and Dengue haemorrhagic fever (DF/DHF) which detecting RDT NS1 and RDT IgM/IgG to assess their performance in a diagnostic laboratory. Sera from 110 patients collected during a febrile outbreak at Chumpae Community Hospital, Khon Kean Province, Thailand during August-December 2010 were studied. The results showed this rapid diagnostic test detected 40 and 23 primary and secondary infective patients respectively. The sensitivity was 63.48 % and specificity was 85.91 % in acute phrase of illness while convalescence phase, the sensitivity and specificity were 83.3 % and 85.81 % respectively. However, the RDT's sensitivity was deviated by the duration after onset of fever. This rapid diagnostic test showed rather low sensitivity because of hospital's delay visit of the patient but showed high specificity in both acute and convalescence phase of illness. The sensitivity was the highest in the early phase of illness (the 3rd - 4th day after onset of illness). By the way, this rapid test would give some sort of benefit by facilitating clinicians to discriminate between primary and secondary infections without the need for expensive equipment or highly trained personnel. In this study, Among the 23 secondary infected child were intensively cared to prevent them from hemorrhagic manifestations.

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APPLICATIONS OF THE ESSENCE DESKTOP SOFTWARE IN THE ANALYSIS OF PHILIPPINE NATIONAL DENGUE DATA

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The recent decades highlight emerging and re-emerging infectious diseases as serious public health threats. There is a need to develop disease surveillance systems able to provide early detection of health threats posing significant risk. The Philippine National Epidemiology Center and the Philippines-AFRIMS Virology Research Unit, Armed Forces Research Institute of Medical Sciences used the open source analysis program, Essence Desktop Edition (EDE) (http://www.jhuapl.edu/Sages/pages/tools/tools.html) developed by Johns Hopkins University-Applied Physics Lab) to analyze CY2003 - 2010 national dengue data collected by the Philippine Integrated Disease Surveillance and Response System. The detection

algorithm in EDE automatically selects linear regression, exponentially-weighted moving average or Poisson regression analysis depending on which algorithm best fits the data. Dengue cases that might signal an outbreak are categorized as "alerts". At the start of May 2010, clusters of red (p value<0.01) and yellow (p value<0.05) alerts were detected, with 134 cases triggering a red alert on May 2. Clusters of alerts were triggered as cases increased to 186 on May 19. From May 11-19, 7 out of 9 days triggered red alerts followed by a steady surge in cases, peaking on August 23 at 1,330 cases. Analysis of dengue cases from the Philippine National Capital Region closely mirrored the 2010 national dengue data time series trend and alerts. A similar predictive trend was seen for the 2009 national dengue data with consecutive red alerts triggered on 9 out of 10 days from May 14-23, followed by a sharp increase in dengue cases. Analysis of 2003-2008 annual dengue data also showed good prediction with cluster of alerts seen approximately 4-6 weeks prior to rapid increase of cases. EDE shows promising applications in early warning alert capability to impending increases in dengue cases in the Philippines. EDE applications are not just limited to disease data but may also have potential applications particularly in analysis of syndromic data.

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EVIDENCE FOR SPATIALLY AND TEMPORALLY CLUSTERED TRANSMISSION AND IMMUNITY OF DENGUE VIRUS FROM HOSPITAL BASED SURVEILLANCE

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Hospital-based surveillance systems are used to characterize the changing patterns of dengue disease incidence over time. These systems often focus on identifying temporal peaks in incidence or spatial hotspots of elevated risk. We demonstrate that by analyzing the distribution of geocoded home addresses of cases over time, these systems can also help us understand the impact of immunity on future case distribution at small spatial scales. All four serotypes of dengue have co-circulated in Bangkok, Thailand, for decades. We characterize the degree to which the homes of dengue cases presenting at a Bangkok hospital between 1995 and 1999 are near each other in space and time. We have found evidence for clustering of cases infected by the same serotype in the same month at distances up to 0.9 km relative to that expected given the overall distribution of cases. This evidence supports the focal nature of dengue dispersal even in a large urban setting. Further, we demonstrate a reduction of homotypic cases with the introduction of temporal lags of greater than 4 months, a reduction of heterotypic cases at lags between 3 and 10 months and an increase in heterotypic cases after 22 months. These clustering patterns are consistent with short term cross-protection between serotypes. At longer time frames, the patterns suggest an increased risk of severe disease from sequential heterotypic infections.

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FOLLOW-UP OF *TRYPANOSOMA CRUZI* RESISTANCE INDUCED BY THE COMPOUNDS BENZNIDAZOLE AND THIOSEMICARBAZONE AND ITS ASSOCIATION WITH P-GLYCOPROTEIN EFFLUX PUMP

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Chagas disease specific treatment is up to now not efficient and presents high toxicity, besides the resistance to the reference drug benznidazole (Bz) for a variety of *T. cruzi* strains. Our group has been focused on the study of the synthetic compound (2-methoxy-styryl)-thiosemicarbazone (2-MEOTIO), a compound that shows trypanocidal activity at concentrations non toxic for the mammal cells. One of the mechanisms related to drug resistance in different pathogenic protozoa is the transport of drugs across the membrane by ATP-binding cassette (ABC) transporters involving P-glycoprotein (Pgp), an efflux pump implicated in multidrug resistance. In the present study, we followed-up the induction of resistance in *Trypanosoma cruzi* epimastigotes to the compounds Bz and 2-MEOTIO and evaluate the possible participation of Pgp in this process. We also investigate the persistence of resistance after morphological transformation to the metacyclic trypomastigote stage. For the trypanocidal activity assay epimastigotes (Y strain) were incubated with Bz and 2-MEOTIO at different concentrations. The IC50 was determined and both drugs were then used to induce resistance in epimastigotes. After 15 passages under drug pressure, it was obtained resistant parasites as demonstrated by a significant increase of the $IC_{s_{50}}$ for both compounds. In order to verify the influence of Pgp, in the mechanism of drug resistance in *T. cruzi,* it was analyzed the efflux of the fluorescent probe Rhodamine 123 (Rho-123) by resistant and wild-type epimastigotes. The assay was realized in the presence/absence of verapamil or cyclosporine A (Pgp inhibitors) and the Rho-123 fluorescence was analyzed on a FACScan flow cytometer. It was observed a significant concentration-dependent reduction of Rho-123 fluorescence in resistant parasites in comparison with wild-type and also the reversion of Rho-123 efflux in the presence of Pgp inhibitors. Metacyclic trypomastigotes were obtained by keeping epimastigotes for 20 days in LIT medium without reposition. The IC50 values for resistant and wild-type trypomastigotes treated with Bz and 2-MEOTIO was then calculated and compared and it was observed an increase of those values. In this work it was demonstrate the participation of Pgp in *T. cruzi* epimastigote resistance induced by Bz and 2-MEOTIO, as well as the persistence of resistance after the differentiation to the metacyclic trypomastigote stage.

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SAFETY AND EFFECTIVENESS OF MEGLUMINE ANTIMONIATE IN THE TREATMENT OF ETHIOPIAN VISCERAL LEISHMANIASIS PATIENTS WITH AND WITHOUT HIV CO-INFECTION

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In sub-Saharan Africa, visceral leishmaniasis (VL) is treated with either PentostamTM (sodium antimony gluconate) or generic sodium stibogluconate (SSG), except in Uganda where Glucantime® (meglumine antimoniate) has been in use for at least a decade. Between January 2008 and February 2009, 54 Ethiopian VL patients were treated with

Glucantime. The medical charts of these patients were reviewed to assess the effectiveness and safety profile of Glucantime in a routine healthcare setting. None of the patients from south Ethiopia (n=24) and 46.4% of the patients from north Ethiopia (n=30) were HIV co-infected. At completion of treatment (Day 31), cure rates were 78.6% (95% CI 59.0-91.7%) in north Ethiopia and 100% (95% CI 85.8-100%) in south Ethiopia. Thirty-three non-serious and six serious adverse events (two pancreatitis, one renal failure and three deaths) were observed in 26 patients. One-third of the non-serious adverse events were due to biochemical pancreatitis. During treatment, a case-fatality rate of 10.0% in north Ethiopia and 0.0% in south Ethiopia was noted. These data show that Glucantime can be as effective as Pentostam or SSG in HIV-negative patients. The data also point to clinical pancreatitis as a safety concern, especially in patients with HIV co-infection.

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THIOSEMICARBAZONES AND SEMICARBAZONES AS POTENT TRYPANOCIDAL AGENTS

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A specific treatment, with more efficiency and less toxicity for Chagas'disease, is the main objective of this study. Thiosemicarbazones and semicarbazones are classes of compounds with medical interest because of their capacity to inhibit the growth of several pathogens. As part of our research program on chemotherapy against diseases caused by trypanosomatids, five thiosemicarbazones and semicarbazones were synthesized, in order to reach a high trypanocidal activity with low toxicity. *In vitro* experiments using *T. cruzi* were carried out to evaluate the effect of those compounds against culture trypomastigotes and amastigotes lodged in both mouse and human macrophages. The enzymatic activity of the nitric oxide synthase (NOS) of the parasite was also evaluated considering that it would be a potential target for those compounds. Besides, the *in vitro* toxicity of those derivatives was evaluated on murine macrophages. In general, thiosemicarbazone derivatives were most effective and among them the 4-N-(2'-methoxy styryl)-thiosemicarbazone was chosen, to compare its *in vitro* effect against amastigotes lodged in both mouse peritoneal and human macrophages. A potent trypanocidal effect of this molecule was observed, more pronounced against parasites interiorized in human macrophages. A potential target in the parasite for this compound was also evaluated by measuring the NOS through NADPH consumption. A significant decrease in the enzyme activity was observed. No macrophage toxicity was observed by any of the compounds, indicating that their activity was specific for the parasite forms investigated. It is important to note the use of human macrophages once these results would be closer to the *in vivo* effect. The significant inhibition of NOS activity is important, considering that this enzyme is a defense mechanism for the parasite allowing its survival within the host macrophage. These data are very promising and a challenge for further studies with these classes of compounds.

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DEVELOPMENT OF NOVEL CHEMOTHERAPY FOR CHAGAS' DISEASE

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Chagas' disease, caused by the parasite *Trypanosoma cruzi*, is a neglected parasitic disease that affects millions of people worldwide mostly residing in Latin America. As a consequence of massive immigration and eco-tourism, numerous patients now live in developed countries including an estimated 1-300,000 in the US. Our group has focused on developing new chemotherapy for Chagas' disease, without the significant toxicity and severe side effects associated to current therapy with benznidazole or nifurtimox. Cruzain (a.k.a cruzipain) and CYP51 are two validated drug targets in the infectious agent *T. cruzi*. Effective irreversible and reversible inhibitors of the major cysteine protease cruzain are available. We identified K11777 as a potent protease inhibitor that rescued animals form lethal infection and characterized its mechanism of action. Briefly, K11777 blocked the autocatalytic processing of the cruzain prodomain inducing accumulation in the Golgi compartment and parasite death. Moreover, K11777 treatment also induced activation of macrophages infected with *T. cruzi* and was cidal for the pathogenic intracellular parasite. We have now identified new derivatives with up to 10-fold more potency than K11777 that are being characterized further and target both cruzain and CYP51 in *T. cruzi*.

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A PROMASTIGOTES RESCUE ASSAY FOR ANTILEISHMANIAL SCREENING OF COMPOUNDS AGAINST INTRACELLULAR *LEISHMANIA DONOVANI* AMASTIGOTES IN THP1 HUMAN ACUTE MONOCYTIC LEUKEMIA CELL LINE

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Leishmania donovani is the causative agents for visceral leishmaniasis (VL), the most fetal form of the disease. The choice of drugs available to treat leishmaniasis is already limited, and even these suffer from limited efficacy and toxicities at therapeutic doses. Most of the first line treatment drugs have already lost their utility due to increasing multiple drug resistance. The current pipeline of antileishmanial drugs is also severely depleted. Sustained efforts are needed to enrich new antileishmanial drug discovery pipeline, which primarily rely on the availability of suitable *in vitro* screening models. The *in vitro* promastigotes and axenic amastigotes assays primarily used for antileishmania drug screening, may not be appropriate due to significant biochemical and molecular differences among these parasite stages compared to intracellular amastigotes. The assays with macrophage- amastigotes models are considered closest to the pathophysiological conditions of VL and are the most appropriate for *in vitro* screening. A promastigotes-rescue assay with transformed THP1 cells infected *in vitro* with *Leishmania donovani* has been developed for screening the pure compounds and natural products extracts and determination of efficacy against the intracellular Leishmania amastigotes. The assay involves controlled lysis of infected macrophages, release of amastigotes and transformation of live amastigotes to promastigotes. The assay compares well with currently used microscopic method, transgenic reporter gene and digital image analysis assays. The assay is highly robust and better compared to microscopic method and measures only the live intracellular amastigotes compared to reporter gene and image analysis assays, which measure both live and dead amastigotes. The assay has

been validated with current battery of antileishmania drugs and has been successful applied for large-scale screening of pure compounds and a library of natural products fractions.

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EVALUATION OF ANTIBODY RESPONSE AGAINST *GLOSSINA* SALIVA IN CATTLE: AN ALTERNATIVE APPROACH TO ASSESS EXPOSURE OF TSETSE BITES

Martin Bienvenu Somda[1], Zakaria Bengaly[1], Emilie Thérèse Dama[1], Anne Poinsignon[2], Sylvie Cornelie[2], Françoise Mathieu-Daude[2], Franck Remoue[2], Antoine Sanon[3], Bruno Bucheton[4]

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Tsetse flies are the notorious transmitters of African Animal Trypanosomosis, the main livestock disease caused by the *Trypanosoma* parasite on the sub-Saharan Africa. Currently, there are not efficient methods to estimate zones at risk or the impact of *Glossina* control campaigns. Therefore, it is important to develop effective tools in order to target the areas where the populations are exposed to a high risk of transmission. The saliva from hematophagous arthropods contains bioactive compounds which play a role in pathogen transmission and induce an immune response into their hosts. Several studies have shown that the antibodies (Ab) against salivary antigens could be used as biomakers of exposure to vector-borne diseases. Our study aims to develop a sero-epidemiological tool to evaluate the cattle exposure to tsetse bites. IgG response against *Glossina* saliva was assessed by ELISA on (i) 101 bovine sera from Burkina Faso of which 48 were sedentary cattle from a tsetse free area and 53 were from a tsetse infested area and (ii) on bovine that were experimentally exposed to tsetse flies and other bloodsucking arthropods. High anti-saliva responses were detected in cows from the tsetse infested area and showed a significantly higher response during the hot dry season. Furthermore, there was a positive association between the anti-saliva response and the risk of being infected by trypanosomes (p=0.03). We have assessed cross-reactions between *Glossina* saliva and other hematophagous vectors. Only the saliva of *Tabanidae spp* induces cross-reactions. In any case, Ab response to *Glossina spp* saliva is transient and decreases within 4 weeks after the stop of experimental exposure. This character is a major advantage to design a biomarker of exposure based on Ab response to tsetse saliva. Immunoproteomic analysis was performed and several specific salivary antigens of *Glossina* were identified. Mass spectrometry is underway. In perspectives, synthetic peptides will be design so as to develop an easy and reproducible test with higher sensitivity and specificity to *Glossina*.

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VALIDATION STUDY OF PCR-MINIEXON FOR DIAGNOSIS OF CUTANEOUS LEISHMANIASIS IN COLOMBIAN PATIENTS

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We evaluated the polymerase chain reaction (PCR) accuracy from Giemsa-stained and methanol-fixed slides from 228 Colombian patients, clinically suspected of having cutaneous leishmaniasis comparing it with a composite reference standard. This reference standard included clinical, histopathological, epidemiological and laboratory criteria. 115 were cases and 113 were non cases according to it. Two samples from cases were excluded from the statistical analysis due to the presence of PCR inhibitors. Patient classification and test application were carried out independently by two blind observers. Miniexon primers were used to amplify a 226-230 bp fragment for *Viannia* subgenus or a 308 bp fragment for *Leishmania amazonensis*; 340 bp fragment for *L. mexicana* or a 418 bp fragment for *L. chagasi*. PCR was positive for 124 samples and negative for 102 samples. All positive samples belonged to *Viannia* subgenus. PCR showed 89.4% (95% CI: 82.4-93.8%) sensitivity and 79.6% specificity (95% CI: 71.3-86%), the positive likelihood ratio was 4.391 (95%CI: 3.03-6.36) and the negative likelihood ratio was 0.133 (95% CI: 0.077-0.229%). The area under the curve in ROC analysis was 0.845 (95% CI: 0.791-0.890) (p<0.0001). 19 of the 23 non cases that showed a positive PCR had an infectious disease (8 gram positive skin infections, 5 allergic reaction to insect bite, 4 sporotrichosis, 1 chromomycosis and 1 myasis) considered in the differential diagnosis. We believe that these false positives may be due to those pathogens and *Leishmania* share miniexon sequences or those patients had mixed infections or these results are mistakes of this assay that impact the specificity. In conclusion, ROC analysis showed that PCR-miniexon from Giemsa-stained and methanol-fixed slides has a good sensitivity and an acceptable specificity in the diagnosis of cutaneous leishmaniasis.

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GENOTYPING OF AMERICAN TEGUMENTARY LEISHMANIASIS USING NON-INVASIVE SAMPLES: PRELIMINARY RESULTS

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Current WHO guidelines recommend genotyping of *Leishmania* as part of the standard management and treatment of American Tegumentary Leishmaniasis (ATL). In Peru, ATL control programs have reduced resources to perform genotyping of *Leishmania* species due to the rural condition of this disease and the limited number of centers enabled to perform the process. Cultures or biopsies have been the most accepted samples to perform genotyping; however, those are invasive, time-consuming and they are not necessarily the best alternatives in terms of tolerability, cost, efficacy and simplification of process. We want to evaluate the usefulness of PCR amplification in lymphatic secretion as a non-invasive sample for the genotyping of ATL. As part of the IDRI-LCVTC-202 LEISH-F2+MPL-SE vaccine trial, genotyping of *Leishmania* by PCR was evaluated in patients coming from endemic areas of *L. (V) peruviana*. Samples were obtained using three methods: biopsies, dermal scrapings and lymphatic secretion. Lymphatic secretion was collected with microhematocrit capillary tubes without any kind of cleaning except debriding of crusty material. Cleaning with Isopropyl pads was performed before dermal scrapings and biopsies were taken with sterile lancets and 2-mm punches. For *L. (V.) peruviana* genotyping, the PCR target was mannose phosphate isomerase gene (MPI), using allele specific primers that distinguish easily between *L. (V.) peruviana* and *L. (V.) braziliensis* or *L. (V.) guyanensis*. Seventeen patients were confirmed as ATL by PCR, scraping or culture of which 14 were identified as *L. (V). peruviana*. The 3 non-identifiable lesions did not have enough DNA to genotype the species. The proportion of samples genotyped by PCR based on each sampling method was as follows: 13 of 17 (76.5%) using biopsies, 11 of 17 (64.7%) using dermal scrapings and 14 of 17 (82.3%) using lymphatic secretion; however, it was not possible to identify statistical differences between methods. In conclusion, the proportion of samples genotypable by PCR using lymphatic secretion may be similar to or even higher than that achieved using invasive samples such as biopsies. We aim to show that genotyping of ATL can be done without invasive procedures while achieving a high performance. These promising results encourage us to conduct better studies to identify differences in diagnostic performance.

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TRANSPORT OF GLUCANTIME® IN HUMAN MACROPHAGES AND ITS INVOLVEMENT IN ANTILEISHMANIAL TREATMENT OUTCOME

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Failure of antileishmanial chemotherapy and the capacity of species of the *Leishmania Viannia* subgenus to cause chronic and persistent infection set the stage for drug tolerant/resistant parasite populations. Host factors are prominently involved in treatment outcome, yet the mechanisms that regulate drug traffiking and metabolism in host cells, and their variation among individuals are unknown. Gene expression profiles of 84 drug transporters were examined in human macrophages following *in vitro Leishmania panamensis* infection and/or exposure to Glucantime®. Ten candidate transporters, including ABC, SLC and AQP family members, whose expression was differentially regulated in response to infection or drug treatment were identified. Transporter gene expression profiles in macrophages from patients who failed or responded to Glucantime® treatment suggest that events of active efflux, reduced uptake, intra-vesicular sequestration and cytoplasmic accumulation, may differentially operate in both patient groups. Downregulation of MRP-1 (GSH-Sb efflux pump) and AQP-9 (an Sb-influx channel) in macrophages from drug responder (n=5) and non-responder (n=7) patients, respectively, strongly suggest a mechanism of transporter-mediated modulation of drug accumulation in host cells from these patient groups. Conversely, ABCB6 and SLC7A11 were upregulated by drug treatment in macrophages from both patient groups suggesting a novel and basic role in intracellular drug mobilization and/or accumulation. Our data shows that *Leishmania* infection and/or drug treatment modulates macrophage transporters for GSH and GSH conjugates, heavy metals and cysteine, strongly suggesting their involvement in transport and detoxification of the drug. These findings provide insights into the mechanisms of drug uptake, efflux and accumulation by the host cells, and variations among individuals that could determine the outcome of treatment.

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SYBR GREEN-BASED QUANTITATION OF *LEISHMANIA* PARASITE LOAD IN LESION BIOPSIES FROM PERUVIAN PATIENTS WITH TEGUMENTARY LEISHMANIASIS

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American tegumentary leishmaniasis (ATL) is a major public health problem in several areas of Latin America. It is characterized by a significant clinical pleomorphism, which has been related to both the infecting *Leishmania* species and the human immune response. Cutaneous leishmaniasis (CL) and mucocutaneous leishmaniasis (MCL) are the main clinical forms of ATL. Real-time quantitative PCR techniques have been utilized for the detection, identification and quantification of New World *Leishmania* species; however, the available techniques generally use expensive labeled probes or lack adequate sensitivity, depending on the target and amplification method used. In this study, we developed a SYBR Green-based real-time quantitative PCR (qPCR) assay to evaluate the *Leishmania* parasite load in Peruvian patients with CL and MCL. Our assay targets the *L. (Viannia)* minicircle kinetoplast DNA (kDNA) that is present at about 10000 copies per parasite. The assay has a linear detection range of 50000 to 0.005 parasite DNA equivalents per reaction. Thirty four lesion biopsies from confirmed CL (n=14) and MCL (n=20) patients were analyzed, among which the parasite numbers ranged from 1 to 144000 per µg of DNA. Patients with CL had significantly higher parasite loads (median 648 parasites/µg DNA) than patients with MCL (median 31 parasites/µg DNA) (P=0.02, Mann-Whitney test). This finding is consistent with earlier observations reported by others, based on histopathology and microscopy of stained tissues. Differences in parasite loads between CL and MCL could reflect the distinct immune responses reported for these clinical forms and warrant further investigation. Our kDNA qPCR assay is highly sensitive and affordable for its implementation in resource-limited settings. It promises to be a useful tool in ATL for studying host-parasite interactions and could be used to guide chemotherapy follow-up and prognosis of disease outcome.

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DEVELOPMENT AND VALIDATION OF A SPECIFIC PCR DIAGNOSTIC PROTOCOL FOR *LEISHMANIA AETHIOPICA*

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Leishmania aethiopica is the main cause of cutaneous leishmaniasis in the Ethiopian highlands, and a growing health burden as more endemic areas

are reported. While the fact that *L. aethiopica* is a clear health threat in Ethiopia, the true endemic extent of the parasite requires further study. Molecular methods, particularly PCR, are commonly used for the detection of *Leishmania* parasites, but species specificity and sufficient sensitivity has often been an issue. Existing protocols specific for *L. aethiopica* required PCR-RFLP, or were not sensitive enough to be clinically applicable. We have developed and validated primers (V5F/V10R) based on the cysteine protease B gene, which is multi copy and polymorphic in *Leishmania*. It is able to differentiate *L.aethiopica* from *L. tropica, L. major, L. donovani, L. infantum*, and *L. chagasi*, and is sensitive enough to detect *L. aethiopica* parasites in biopsy samples alone. These primers have the potential to be extremely useful in a clinical setting for rapid diagnosis of cutaneous leishmaniasis. Additionally, their use in epidemiological studies may aid in better knowledge of the true prevalence and impact of *L. aethiopica* in East Africa.

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A COMBINED DNA VACCINE AGAINST *TRYPANOSOMA CRUZI* REDUCES CARDIAC INFLAMMATION IN DOGS

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American trypanosomiasis is a major neglected public health problem in America. No vaccines are available for the disease. DNA vaccine represents an alternative technology to evaluate, as they induce mainly a Th1 immune response; Th1 responses are needed to control *Trypanosoma cruzi* infections. In this study we tested a DNA vaccines encoding TSA-1 and Tc24; two plasmid construction -that have proven its potential in murine models- in dogs during the acute phase (60 days post-infection). 6 healthy stray dogs were vaccined intramuscularlly with 250µg of each plasmid at days -28 and -14 of infection, while 5 received 500µg of empty plasmid (PcDNA3) in the same regimen. Dogs were experimentally infected with 2000 metacyclic trypomastigotes per kg via intraperitoneal, then, clinical, immunological and pathological features were followed the next 60 days. 5 of 5 dogs of control group developed cardiac arrythmias while only 2 of 6 vaccinated dogs did, but differences did not reach significance (p=0.065; log rank test). Dogs developed a very low parasitemia and no significant differences were found between groups along the observation time. No differences were found for IgG, and isotypes IgG2a and IgG1 between groups either. Cardiac inflammation was significantly lower in vaccinated dogs (p=0.0303; Mann-Whitney test). Lymphocyte counts were significantly higher for vaccinated dogs at day 28 post-infection (p=0.0215; T). Hematocrit was significantly lower in control dogs by day 28 post-infection (p=0.0149; T). Although this DNA vaccine could not avoid infection, and did not alter parasitemia its beneficial effects were observed reducing cardiac inflammation, this effect might be due to a control of the immune system of *T. cruzi* infection, this also led to a minor development of cardiac arrhythmias. Lymphocyte counts may be evidence of an induction of immunity as vaccinated dogs showed higher counts and developed lower degree of inflammation. Control of chronic inflammation was also reflected by higher hematocrit in vaccinated dogs by day 28. Parasitic load is to be measured by qPCR. This results strongly indicate that DNA vaccination has improved the immune response against *T. cruzi* infection during the acute phase in dogs.

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THE NATURAL PRODUCT CYNAROPICRIN INHIBITS *TRYPANOSOMA BRUCEI RHODESIENSE* IN THE ACUTE MOUSE MODEL

Stefanie Zimmermann[1], Michael Adams[1], Reto Brun[2], Matthias Hamburger[1]

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In a medium throughput screen of 1800 plant and fungal extracts for antiplasmodial, antitrypanosomal and leishmanicidal activity, a dichloromethane extract of *Centaurea salmantica* L. (*Asteraceae*) showed strong inhibition of *Trypanosoma brucei rhodesiense*. The active constituent was shown to be a guajanolide sesquiterpene lactone. Against *T. brucei rhodesiense* cynaropicrin had an IC_{50} of 0.3 µM. It was ten and fifteen times less active against *Plasmodium falciparum* (IC_{50}: 3.0 µM) and *T. cruzi* (IC_{50}: 4.4 µM), respectively. In a primary *in vivo* study with six mice infected with *T. b. rhodesiense* bloodstream forms were treated daily with 5 and 10 mg/kg bid cynaropicrin i.p. for three consecutive days. When treated with 10 mg/kg bid the mice showed 92% decreased parasitemia on day 7 postinfection. The test animals died of the infection on day 12, which is 9 days after the termination of the treatment, whereas the control animals died within six days. This is the first study of a natural product showing *in vivo* activity against *T. b. rhodesiense*. Preliminary structure activity studied will be shown.

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EFFICACY OF RADIO-FREQUENCY INDUCED HEAT THERAPY VESUS INTRALEISONAL SODIUM STIBOGLUCONATE TREATMENT IN LOCALIZED CUTANEOUS LEISHMANAISIS CAUSED BY *LEISHMANIA TROPICA* IN INDIA

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Localised cutaneous leishmaniasis (LCL) is a wide spread protozoal infectious disease caused by *Leishmania* parasite. LCL is endemic in the Bikaner, India and causative agent being L. tropica. In search of

a well tolerated, effective therapy with good compliance, we used radiofrequency heat therapy (RFH) and compared it with twice weekly intralesional sodium stibogluconate (SSG). One Hundred fresh established cases of CL were included in the present study. Alternate patient were categorized into two groups, Group A and B of 50 each. GroupA patients were treated with RFH (50°C for 30 seconds) once. Group B patients were given seven, twice weekly intralesional SSG in dosage of 50mg/cm2 of lesion. Lesions were evaluated at 6th, 8th, 10th 12th, 16th, 20th and 24th weeks. RFH and intralesional SSG injection were well-tolerated. Complete cure rate of lesions at 6th, 8th, 10th, 12th and 20th weeks were 24%, 42%, 50%, 82% and 98% in group A and 30%, 44%, 56%, 76% and 92% in group B respectively. In conclusion, both modalities are effective and well-tolerated. Intralesional injections of SSG are painful, cause localized edema, requires several visits, whereas RFH is a ruggedized, non invasive, painless, battery operated method, requires single session, cosmetically more acceptable. So, RFH is better alternative to intralesional injections of SSG in resource poor country like India.

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INTERFERON-Γ RELEASE ASSAY (MODIFIED QUANTIFERON) AS A POTENTIAL MARKER OF INFECTION FOR *LEISHMANIA DONOVANI*, A PROOF OF CONCEPT STUDY

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In areas endemic for visceral leishmaniasis (VL), a large number of infected individuals mount a protective cellular immune response and remain asymptomatic carriers. We propose an interferon-γ release assay (IFN-γRA) as a novel marker for latent L*eishmania donovani* infection. We modified a commercial kit (QuantiFERON) evaluating five different leishmania-specific antigens; H2B, H2B-PSA2, H2B-Lepp12, crude soluble antigen (CSA) and soluble leishmania antigen (SLA) from *L. donovani* with the aim to detect the cell-mediated immune response in VL. We evaluated the assay on venous blood samples of active VL patients (n=13), cured VL patients (n=15), non-endemic healthy controls (n=11) and healthy endemic controls (n=19). The assay based on SLA had a sensitivity of 80% (95% CI= 54.81-92.95) and specificity of 100% (95% CI= 74.12-100). Our findings suggest that a whole-blood SLA-based QuantiFERON assay can be used to measure the cell-mediated immune response in *L.donovani* infection. The positive IFN-γ response to stimulation with leishmania antigen in patients with active VL was contradictory to the conventional finding of a non-proliferative antigen-specific response of peripheral blood mononuclear cells and needs further research.

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LYMPH NODE DYSFUNCTION IN EXPERIMENTAL MODEL OF MALNUTRITION AND VISCERAL LEISHMANIASIS

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Undernutrition is a key factor for the development of visceral leishmaniasis (VL). However, the mechanistic relationship between malnutrition and the course of VL at the immunological level is poorly understood. In a murine model of polynutrient (protein, iron and zinc) deficiency that resembled moderate human malnutrition in children we found that malnutrition led to increased early visceralization following cutaneous *Leishmania donovani* infection. This increased early visceralization was related to altered lymph node barrier function so we sought to investigate the mechanism by which this occurs. Well-nourished (control) and malnourished mice were inoculated intradermally in the footpad with *L. donovani* promastigotes and the popliteal lymph nodes harvested 3 days post-infection. There was no gross difference in the lymph node histopathology or in the proportion or localization of endothelial cells, fibroblast reticular cells (FRC), B cells, or T cells between the infected malnourished and well-nourished groups as demonstrated by immunohistochemistry and flow cytometry. However, by flow cytometry the total number of DC, neutrophils and macrophages was significantly less in the lymph node of malnourished *L. donovani* infected mice compared with the well-nourished controls ($p<0.01$). With PCR array and real-time PCR techniques, we found that malnourished infected mice showed significant down-regulation of the expression of a group of genes that have been shown to play a role in dendritic cell chemoattraction and retention: chemokine (C-C motif) ligand 2 (CCL2), CCL7, CCL11, chemokine (C-C motif) receptor 2 (Ccr2), C-X-C motif chemokine 10 (CXCL10), interferon- γ, and secreted phosphoprotein 1 ($p<0.05$ for all). Notably, CCL2, CCL7, and CXCL10 are known to be produced by FRC in the lymph node. These results suggested that the impaired capacity of the lymph node to act as a barrier to dissemination of *L.donovani* infection is accompanied by dysregulation of the molecular signals involved in cellular trafficking and retention in the lymph node.

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CD8+CD28- T CELLS FROM CHRONIC CHAGASIC PATIENTS INVOLVED IN MYOCARDIC DESTRUCTION

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Human infection with *Trypanosoma cruzi* leads to Chagas disease, which presents as several different clinical phenotypes ranging from an asymptomatic form to a severe dilated cardiomyopathy. Several groups have demonstrated that T cells play a critical role in cardiac pathology as well as in immunoregulation during chronic disease. Given that CD28-negative T-cells are so frequent in chagasic patients and considering the precise phenotype, function and specificity of these cells remain elusive, this study was designed to better characterize CD28- cellular subsets in Chagas disease context. Patients from the polarized forms, indeterminate and severe cardiopathy, were carefully selected and screened for CD28 expression in peripheral blood by flow cytometry. Disease activity correlated with lack of CD28 expression since CD28+ cells were significantly less frequent in patients with severe cardiac damage. Cellular analysis with regards to their activation, migration and cytotoxic potential were performed by flow cytometry after *in vitro* stimulation with cardiac and parasite antigens. Our results show that CD4+CD28- T cells from chagasic patients display a phenotype related to effector functions (high expression of CD11a, HLA-DR and granzyme A). Previously, we have shown a correlation between CD4+CD28- cells from indeterminate and cardiac chagasic patients and the expression of IL-10 and TNF-α, correspondingly. These data suggest that CD4+CD28- T cells from indeterminate and cardiac patients, despite of their similar characteristics, exert their activities differently in asymptomatic or symptomatic patients, controlling or exacerbating the disease, respectively. Previous data showing a high frequency of CD8+ cells in chagasic myocardial tissue in association with our results showing CD8+CD28- T cells as activated, ended differentiated, able to migrate and having enhanced cytotoxic ability in a heart/parasite antigens environmental, might indicate that CD8+CD28- T cells are important effector cells in lesion site causing cardiac tissue damage observed in Chagas disease.

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IMMUNOLOGICAL PROFILE OF ASYMPTOMATIC LEISHMANIASIS INDIVIDUALS AFTER VISITING ENDEMIC AREAS IN PERU

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People who visit Leishmaniasis endemic areas could be exposed to the parasite but would not necessarily present any clinical manifestation. We decided to assess immune factors contribution to disease outcome. Because host immune response plays a central role in determining disease outcome, we investigated the cellular and humoral immune response in asymptomatic leishmaniasis patients. Sera and blood were collected from individuals with cutaneous (CL; 7), mucosal (ML; 12) leishmaniasis and those without any sign of the disease (28). The control healthy subjects (HS; 8), never visited endemic areas. Asymptomatic (ASY) were defined as individuals who showed T-cell proliferation with no disease. They represented 54% of those who visited endemic areas (SI median=4.70). ASY presented low levels of IFNγ, TNFα and IL10 (medians= 18.60, 6.83 and 31.50, respectively), but a remarkably low IFNγ/IL10 ratio (median=0.32) ($p<0.05$, when compared with HS). ELISA for IgG isotypes showed that IgG3 and IgG1 were detected in 40% and 6.7% of ASY respectively. These levels of cytokines and IgG isotypes were considerably lower than corresponding values from CL/ML patients ($p<0.01$). Exacerbated pro-inflammatory response were found in CL and ML (Medians: SI=77.60 and 22.20; IFNγ=3259.48 and 4,673.70; TNFα=66.30 and 155.90; IFNγ/IL10 ratio=61.27 and 64.30) respectively. IgG1 and IgG3 were detected in most of the CL/ML samples (100% and 83.3%). Immune response in ASY was characterized by both, moderate cell proliferative response and production of IFNγ and TNFα when compared with CL/ML, despite similar IL10 production in these groups. Furthermore, it is interesting to note the presence of IgG3 and absence of IgG1 in ASY, whereas both are present in CL/ML patients. This fact might suggest that other factors different than IL10 could be involved in the modulation of Th1 response in ASY.

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FIRST REPORT OF NATURAL *LEISHMANIA* INFECTION OF *LUTZOMYIA AURAENSIS* IN MADRE DE DIOS, PERU, DETECTED BY A NOVEL REAL-TIME PCR ASSAY

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Leishmania species of the Viannia subgenus are responsible for most cases of New World leishmaniasis (NWL) in South America. Studying the prevalence and distribution of *Leishmania*-infected vectors is critical to understanding the dynamics of disease transmission and predicting the emergence of new endemic areas. In the year 2010 up to 6 761 new cases of leishmaniasis were reported in Perú. We used a novel real-time PCR assay to detect natural *Leishmania* infections in phlebotomines collected in ten households from a jungle community in Madre de Dios, Peru. Using miniature CDC light traps, we collected a total of 1299 female sand flies belonging to 33 species. *Leishmania* genus was detected by kDNA PCR and species were identified by real-time PCR of the genes 6PGD and MPI that allows the differentiation of up to 6 *Leishmania* species. Seven out of 164 pools (4.3%) were positive for *Leishmania*. We identified four positive pools of *Lutzomyia auraensis*, three with *Leishmania Viannia lainsoni* and one with *Leishmania Viannia braziliensis*. Our findings revealed a large predominance of *Lu. auraensis*, which comprised 63% of all collected sand flies in the study area and its minimal infection prevalence was conservatively calculated at 0.6% (5/821). Other sand fly species infected with *Leishmania Viannia braziliensis* were *Lutzomyia davisi, Lutzomyia punctigeniculata* and *Lutzomyia trinidadensis*. This novel real-time PCR assay allowed us to implicate for the first time *Lutzomyia auraensis*, a common sand fly in this region of the Amazon Basin, as a new carrier of pathogenic species of *Leishmania*. Further studies are needed to assess the importance of *Lutzomyia auraensis* and other sand fly species in the transmission of NWTL in hyperendemic areas of Peru.

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FUNCTIONAL CHARACTERIZATION AND GENETIC DISRUPTION OF IMPDH GENES IN *TRYPANOSOMA CRUZI*

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Trypanosoma cruzi is incapable of de novo purine biosynthesis and is dependent on the salvage of exogenous purines for growth. Inosine monophosphate dehydrogenase (IMPDH) is responsible for the rate-limiting conversion of inosine monophosphate to xanthine monophosphate in the synthesis of guanine nucleotides. The objective of this study was to evaluate the function and essentiality of *T. cruzi* IMPDH enzymes for parasite growth and viability. Two IMPDH enzymes, *TcIMPDH1* and *TcIMPDH2*, have been annotated in the *T. cruzi* genome, and transcriptome analysis of the four parasite life stages reveals high expression of both enzymes in amastigotes. Phylogenetic analysis demonstrated these TcIMPDHs group with other eukaryotic and

prokaryotic IMDPH proteins; interestingly, *TcIMPDH2* clustered with the *Cryptosporidium parvum* IMPDH, a gene apparently obtained in *C. parvum* by lateral transfer from an epsilon-proteobacterium. Bacterial complementation of IMPDH-deficient *E. coli* strains confirmed IMPDH activity of the *TcIMPDH1* gene product, however, no growth of *TcIMPDH2*-bearing colonies suggested an alternative function of *TcIMPDH2* gene products. The *Leishmania major* gene syntenic with *TcIMPDH2* was recently re-annotated as a guanosine monophosphate reductase (GMPR), so we asked if the *TcIMPDH2* might also be a GMPR. However, testing in a GMPR-deficient *E. coli* strain revealed no GMPR activity for either *TcIMPDH1* or *TcIMPDH2*. To further investigate the role of TcIMPDH2, we generated parasite lines where the gene was selectively disrupted by homologous recombination using a drug resistance gene. *TcIMPDH2* null parasites grew normally as epimastigotes and converted to metacyclic trypomastigotes, which were infective in C57BL/6 mice as evidenced by the induction of a strong *T. cruzi*-specific $CD8^+$ T cell response. Currently, we are developing *TcIMPDH1* KO parasites to compare the role of these two apparently functionally distinct genes. Our results confirm the function of *TcIMPDH1* while suggesting no IMPDH or GMPR function for the gene annotated as *TcIMPDH2*. Successful construction of null *TcIMPDH2* parasites suggests this gene is not essential for *T. cruzi*.

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TRYPANOSOMA CRUZI INTERACTS WITH HUMAN ADIPOCYTES AND ITS INFECTION IN HUMAN AND MICE RESULTS IN DIFFERENTIAL SERUM APOLIPOPROTEIN A1 PROFILE

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Chagas disease (CD) is caused by the protozoan parasite, *Trypanosoma cruzi*. Endemic in Central and South America where ~17 million persons are infected, latent infections can persist for decades, causing terminal, cardiomyopathy in ~30% of subjects. According to previous observations, CD patients, even those who die from cardiac complications have a lower incidence rate of atherosclerosis. However, levels of HDL and Apolipoprotein A1 (Apo A1) in CD patients are reported to be normal using ELISA. Recently, our laboratory discovered several novel biomarkers for CD using mass spectrometry (1). We have identified intact Apo A1 as a negative biomarker for CD and several truncated forms of Apo A1 as positive biomarkers for CD. Apo A1 is the principle protein found in high density lipoprotein (HDL). We intended to validate our human Apo A1 findings in the CD1 acute/chronically-infected mice model. However, we demonstrated that murine Apo A1, though sharing approximately 60% similarity with human Apo A1, did not share the same cleavage patterns with human Apo A1. In contrast, Apo A1 in infected mice serum formed several high molecular weight complexes. Adipocytes have recently come under the spotlight of CD research as the reservoir of *T. cruzi* during chronic infections. Because adipocytes and HDL are both major players in the host lipid metabolism and they closely interact with each other, we decided to investigate *T. cruzi* infections in the human adipocyte system. We utilized cultured primary human adipocyte as our *in vitro* model. We discovered that human adipocytes ameliorate *T. cruzi* infection based on the number of *T. cruzi* amastigotes housed by the adipocytes 3-day post infection and confirmed the result using real time PCR. As well, *T. cruzi* interacts closely with cellular lipid storage as observed by light microscopy. The phenomenon will be observed more closely by fluorescent microscopy. Our findings may shed light on the interaction of *T. cruzi* and human host system during the chronic infection stage. It may also uncover the impact of *T. cruzi* on host lipid homeostasis.

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EVALUATION OF THE AMASTIGOTE-SPECIFIC MITOCHONDRIAL MEMBRANE PROTEIN ENCODING GENE DELETED PARASITE AS A LIVE ATTENUATED VACCINE CANDIDATE AGAINST LEISHMANIASIS

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Leishmaniasis causes a significant morbidity and mortality worldwide. There is no vaccine available against leishmaniasis. Various approaches to develop a vaccine against leishmaniasis have had limited success so far. In this study, we are evaluating the use of live attenuated parasites as vaccines. *Leishmania donovani* parasites that are deleted for the amastigote specific protein p27 gene (Ldp27), which is a component of an active cytochrome c oxidase complex, were used as a vaccine in the mouse and hamster models. Ldp27 gene deleted parasites do not survive more than 13 weeks in BALB/c mice or hamsters and do not cause any pathogenesis as indicated by histological analysis of the liver. Immunization with p27 gene deleted parasites for 8 weeks and challenge with wild type virulent *L. donovani* for 12 weeks, showed a significantly lower spleen and liver parasite burden compared to non-immunized mice post challenge. Protection in immunized mice was correlated with the stimulation of multifunctional Th1 type CD4 cells after challenge with the wild type parasite. Mice immunized for 16 weeks, when no p27 gene deleted parasites are observed, and challenged with virulent parasite showed significant reduction in parasite burden post challenge suggesting the participation of memory T cell response in protection from infection. Currently we are investigating the mechanism of memory cell response to live *L. donovani* attenuated parasites in mice. We are also evaluating the vaccine potential of p27 gene deleted parasites in hamsters, a model for leishmaniasis disease.

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AN APPROACH TO DETERMINE THE TRANSCRIPTOME OF *TRYPANOSOMA BRUCEI RHODESIENSE* FROM SLEEPING SICKNESS PATIENTS IN UGANDA

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Trypanosoma brucei rhodesiense is a protozoan parasite that causes the acute form of Human African trypanosomiasis, commonly referred to as Sleeping Sickness. The disease is characterized by two stages, the early haemolymphatic stage and late meningoencephalitic stage, which leads to death if untreated. So far all expression profiling studies of African trypanosomes have been carried out on cultured parasites or high-density mouse infections. However, we have no idea to what extent these parasites are representative of a real human infection. My work is therefore aimed at analyzing the transcriptomes of clinical isolates of *T. b. rhodesiense* from patient peripheral blood and cerebral spinal fluid, using high throughput RNA sequencing technology. Furthermore the genomes from these infective parasites will be sequenced and analyzed for structural variations and possible drug resistance markers. But given the low parasitaemia during active infection, there is a need to amplify the trypanosome RNA above the human cellular RNA background. Hence, using the spliced leader sequence that is attached to the 5' end of all trypanosome mRNAs following *trans*-splicing, I am developing a technique to specifically amplify nanogram concentrations of trypanosome RNA against a background of microgram amounts of human RNA.

Subsequently, the method will be used on patient samples to analyze the transcriptomes of trypanosomes from blood and CSF for possible differential gene expression. Furthermore we would like to know whether the parasites from human infections differ from those cultured in the laboratory.

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MULTILOCUS GENOTYPING REVEALS A POLYPHYLETIC PATTERN AMONG NATURALLY ANTIMONY-RESISTANT *LEISHMANIA BRAZILIENSIS* ISOLATES FROM PERU

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Pentavalent antimonials (Sb^V) remain the mainstay treatment against leishmaniasis worldwide, but their efficacy is threatened by the emergence of drug-resistant parasites, as described in several endemic regions. In order to understand the epidemiological dynamics of Sb^V resistance in zoonotic tegumentary leishmaniasis and its link with treatment outcome, we analyzed the population structure of 24 Peruvian *Leishmania braziliensis* clinical isolates with known *in vitro* antimony susceptibility and clinical phenotype by multilocus microsatellite typing (14 microsatellite loci). The genetic variability as defined by the used loci in the Peruvian isolates was high and the multilocus genotypes were highly divergent from each other. No association was found between the genotypes and *in vitro* drug susceptibility or clinical treatment outcome. These findings, together with the polyphyletic pattern shown by the Sb^V-resistant *L. braziliensis* parasites in the Neighbour-Joining dendrogram might be explained by (i) independent events of drug resistance emergence, (ii) sexual recombination and/or (iii) other phenomena mimicking recombination signals. Interestingly, the polyphyletic pattern observed here is very similar to the one we observed in the anthroponotic *L. donovani,* as reported previously, hereby questioning the role of transmission and/or chemotherapeutic drug pressure in the observed population structure.

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IDENTIFICATION OF SYNTHETIC PEPTIDES FROM *GLOSSINA PALPALIS GAMBIENSE* SALIVA AS BIOMARKER CANDIDATE OF EXPOSURE

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Vertebrates have three efficient systems that make life potentially difficult for hematophagous animals: hemostasis, inflammation, and immunity. These three complex physiological responses interact with each other in order to counteract the host's barriers by using a complex mixture of pharmacologically active components, which are injected into the host skin during the probing and ingestion phases of feeding. In addition, some salivary proteins are immunogenic and can initiate a specific antibody (Ab) response that could be a potential marker of exposure to vector-borne diseases in individuals exposed to bites of arthropod vectors. The first objective of this work was to assess if the IgG response directed against *Glossina* saliva was representative of the human-tsetse contact. For this purpose, saliva from Gpg was collected by an experimental procedure, and reactivity of human plasma from active HAT foci (Guinée) and no infested foci (Burkina) was evaluated by indirect ELISA. Though the highest anti-saliva responses have been observed in the HAT foci of Guinea in contrary to Bobo and Loropeni (non infected foci), a low specificity of this marker have been noted. This is why, secondly we aimed to improve this tool by indentifying synthetic peptides from Gpg saliva to substitute whole saliva for its. To accomplish this purpose, we realized 2D gel electrophoresis follow up to blots with 2 pools of exposed and unexposed individuals' plasmas. Blots alignment by the Samespots software followed by mass spectrometry (MS, LCMS/MS) analysis allowed identifying of seven proteins, all in Gmm and in which three were specific: adenosine deaminase (41KDa), Tsetse Saliva Growth Factor (56-58KDa), and antigen 5 (29KDa). Bioinformatic analysis using epitopes prediction software and alignment Blast led us to target 3 sequences from these three proteins that can be use as good biomarker candidates. In the next stage, the potential biomarker of these peptides will be assessed by ELISA in order to retain the peptide that will give the best result.

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IFN-Γ AND IL-10 SEQUENCES AND EXPRESSION ANALYSIS IN *PEROMYSCUS YUCATANICUS*

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In southeast Mexico, *Peromyscus yucatanicus* is the primary reservoir of *Leishmania (Leishmania) mexicana,* main agent of localized cutaneous leishmaniasis (LCL). We have already demonstrated that this deer mouse reproduced both clinical and subclinical infections by *L. (L.) mexicana* similar to those of humans as well as production of nitric oxide in response to infection. We have also proposed to use *P. yucatanicus* as the experimental model to characterize the immune response to *L. (L.) mexicana* but immunological tools remain lacking. Thus, the aim of this study was to sequence INF-γ and IL-10 of *P. yucatanicus* in order to analyze their expression. These cytokines were amplified by RT-PCR using *P. maniculatus* primers. Partial cDNAs were cloned into p-GEMT Easy and sequenced. The INF-γ was 240 nucleotides long and shared 96% nucleotide identity with *P. maniculatus*. IL-10 sequence had 365 nucleotides and shared 84% nucleotide with *P. maniculatus*. RT-PCR analysis of splenocytes stimulated by Concanavalin A determined that maximal INF-γ expression was at 8 hour however, no peak happened during IL-10 expression. Results supported the use of *P. maniculatus* primers to study the immune response in *P. yucatanicus* infected by *L. (L.) mexicana.*

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TRANSPLACENTAL TRANSMISSION OF *LEISHMANIA INFANTUM* WITHOUT IMMUNOLOGIC TOLERANCE AS A MEANS FOR CONTINUED DISEASE INCIDENCE IN NORTH AMERICA

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Dogs are the predominant domestic reservoir for human *L. infantum* infection. Zoonotic visceral leishmaniasis (ZVL) is an emerging problem in some U.S. dog breeds, with an annual quantitative PCR prevalence of greater than 20% within an at-risk canine population. Classically *Leishmania* is transmitted by infected sand flies and phlebotomine sand flies exist in the United States, means of ongoing *L. infantum* transmission in U.S. dogs is currently unknown. Possibilities include vertical (transplacental/transmammary) and horizontal/venereal transmission. Several reports have indicated that endemic ZVL may be transmitted

vertically. Our aims for this present study were to establish whether vertical/transplacental transmission was occurring in this population of *Leishmania*-infected US dogs and determine the effect that this means of transmission has on immune recognition of *Leishmania*. A pregnant *L. infantum*-infected dam donated to Iowa State University gave birth in-house to 12 pups. Eight pups humanely euthanized at the time of birth and four pups and the dam humanely euthanized three months post-partum were studied via *L. infantum*-kinetoplast specific quantitative PCR (kqPCR), gross and histopathological assessment and CD4+ T cell proliferation assay. This novel report describes disseminated *L. infantum* parasites as identified by kqPCR in 8 one day old pups born to a naturally-infected, seropositive U.S. dog with no travel history. Despite presence of disseminated parasites, pups had a productive T cell proliferative response to parasite antigen at a day of age, also present at 12 weeks old, indicating absence of immunologic tolerance despite *in utero* infection. This is the first report of vertical transmission of *L. infantum* in naturally-infected dogs in North America, emphasizing that this novel means of transmission could possibly sustain infection within populations. Evidence that vertical transmission of ZVL may be a driving force for ongoing disease in an otherwise non-endemic region has significant implications on current control strategies for ZVL, as at present parasite elimination efforts in endemic areas are largely focused on vector-borne transmission between canines and people. Determining frequency of vertical transmission and incorporating canine sterilization with vector control may have a more significant impact on ZVL transmission to people in endemic areas than current control efforts.

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IMMUNOLOGICAL BIOMARKERS FOR THE DETECTION OF LOW-DENSITY INFESTATIONS OF *TRIATOMA INFESTANS* TO SUPPORT CHAGAS DISEASE CONTROL

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Early detection of low density or re-emerging triatomine populations is critical if the efficacy of Chagas disease control programmes is to be maintained. We developed an immunological monitoring approach that can be applied to measure low infestation and re-infestation of *Triatoma infestans* and other reduviid bugs. IgG and IgM antibody responses of guinea pigs and/or chickens to the saliva of *T. infestans* revealed significant differences between sera from animals exposed to low and high numbers of triatomines in the laboratory or field. We identified a highly immunogenic 14.6 kDa salivary protein of *T. infestans* and synthesised a recombinant form (rTiSP14.6) of the antigen. rTiSP14.6 was highly effective for detecting differences in exposure levels of *T. infestans* using IgG and IgM antibody responses of sera from laboratory-exposed and field-exposed chickens from households in Bolivia with low and high infestation levels of *T. infestans*. rTiSP14.6 was also confirmed as a sensitive exposure marker for at least four further triatomine species but it did not cross react with anti-saliva antibodies elicited by unrelated haematophagous arthropods. Differential analysis for IgG and IgM components of the anti-saliva response allowed the detection of very recent exposures due to the short persistence of the IgM response, and thus offers a method to detect re-infestation of triatomines even shortly after insecticide based control programmes have been completed.

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HOST CANDIDATE GENE POLYMORPHISMS AND CLEARANCE OF DRUG-RESISTANT *PLASMODIUM FALCIPARUM* PARASITES IN AFRICA

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The correlation of treatment outcome and molecular tests is not perfect, due in part to individuals who are able to clear drug-resistant parasites. This study aimed to refine and validate molecular markers in the human genome that correlate with the clearance of malaria parasites after specific treatment, despite the drug resistance profile of the protozoan as determined by molecular approach. 4541 samples from six African countries which were known to contain drug resistant parasites were analysed. These parasites were collected from patients who subsequently failed to clear or not their infection following drug treatment, as expected. 4418 samples were successfully analysed, using Sequenom's mass spectrometry iPLEX gold platform, for 67 human polymorphisms (SNPs) on 17 chromosomes, to identify regions of the human genome which contribute to enhanced clearance of drug resistant parasites. An analysis of all data from the six countries revealed significant associations between the phenotype of ability to clear drug-resistant *Plasmodium falciparum* infection and two human immune response loci common to all populations. Overall, two SNPs showed a significant association with clearance of drug-resistant parasites with Odds Ratios of 0.76 (95% CI 0.62-0.92, P= 0.005) and 0.67 (95% CI 0.45-0.99, P= 0.046). The first SNP (located at 5q31-33) has previously been reported to be involved in the control of malaria parasite density, and the locus contains genes encoding Th2 cytokines such as IL-4, IL-5 and Il-13.The second SNP (on chromosome 22) occurs within a Der1-like domain family gene, and linkage to this locus has not previously been reported in studies of malaria. The study showed significant association of three loci in the human genome with the ability of parasite to clear drug-resistant *P. falciparum* in samples taken from six countries distributed across sub-Saharan Africa. One locus has previously been linked to the control of malaria parasite density, and it is possible that patients able to clear drug-resistant infections have an enhanced ability to control parasite growth. Two loci are involved in the Th1/Th2 balance, and the association of SNPs within these genes suggests a key role for antibody in the clearance of drug-resistant parasites. The other locus encodes a protein involved in the degradation of misfolded proteins within the endoplasmic reticulum, and its role, if any, in the clearance phenotype is unclear.

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MALARIA INFECTED ERYTHROCYTES STIMULATE MONOCYTE-DERIVED MACROPHAGE INFLAMMATORY CYTOKINE PRODUCTION WHICH IS IMPAIRED BY HIV INFECTION

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Women in first pregnancy lack protective immunity to *Plasmodium falciparum* and are at increased risk of severe anaemia and low infant birth weight associated with monocyte infiltrates in the maternal circulation of the placenta. Antibody and complement opsonise infected erythrocytes (IE) sequestered in the placenta resulting in macrophage and monocyte phagocytic clearance. Production of pro-inflammatory cytokines and β chemokines by macrophages and monocytes cause an alteration in the placental cytokine balance. This immune response leads to both phagocytosis of IE which restricts infection and promotes antibody-mediated immunity and cytokine production which causes immunopathology and poor outcomes. We hypothesise that modulation of phagocytic function by antibody, complement and acquired cellular immunity is a key determinant of the balance between host protection and immunopathology in malaria infection. In addition, HIV infection increases the risk and severity of pregnancy-associated malaria by poorly defined mechanisms. The aim of this study was to measure human monocyte-derived macrophage (MDM) cell signaling events, inflammatory cytokine mRNA and secretion following exposure to IE opsonised with pooled patient sera with and without HIV-$1_{Ba\text{-}L}$ co-infection. IL-6 transcript and secreted protein were detected following stimulation by unopsonised CS2-IE in the absence of ingestion suggesting internalization is not required. Ongoing work involves determining the pattern recognition receptor responsible. In contrast, IL-1β and TNFα mRNA but not secreted protein was detected in response to IE. Antibody opsonisation resulted in ingestion, and in IL-6, IL-1β and TNFα transcription and secretion. Interestingly, the NF-κB pathway was activated in response to both opsonised and unopsonised CS2-IE. *In vitro* HIV infection reduced MDM phagocytosis and pro-inflammatory cytokine mRNA transcription and secretion. Thus inadequate phagocytosis and cytokine secretion in the context of HIV infection may reflect the poor immune response to parasitemia.

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ELEVATED PLASMA VON WILLEBRAND FACTOR AND PROPEPTIDE LEVELS IN MALAWIAN CHILDREN WITH MALARIA

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In spite of the significant mortality associated with *Plasmodium falciparum* infection, the mechanisms underlying severe disease remain poorly understood. We have previously shown evidence of endothelial activation in Ghanaian children with malaria, indicated by elevated plasma levels of both von Willebrand factor (VWF) and its propeptide. In the current prospective study of children in Malawi with mild and complicated malaria, we investigated the specificity of these markers for malarial disease, using the presence of retinopathy as an indicator that coma in an individual is likely to be due to *P. falciparum* infection. Children with cerebral malaria, mild malaria and controls without malaria were recruited into the study. All comatose patients were examined by direct and indirect ophthalmoscopy. Plasma VWF and propeptide levels were measured by ELISA. Mean VWF and propeptide levels were higher in patients with uncomplicated malaria than in children with non-malarial fevers of comparable severity, in whom mean levels were higher than in non-febrile controls. Mean concentrations of both markers were higher in cerebral malaria than in uncomplicated malaria, and were similar in patients with and without retinopathy. Levels of both VWF and propeptide fell significantly 48 hours after commencing therapy and were normal one month later. In conclusion, plasma VWF and propeptide levels are markedly elevated in both cerebral and mild paediatric malaria, with levels matching disease severity, and these normalize upon recovery. High levels of both markers also occur in retinopathy-negative cerebral malaria cases, many of whom are thought to be suffering from diseases other than malaria, indicating that further studies of these markers are required to confirm their specificity.

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POST-MORTEM ANALYSIS OF *VAR* GENE GROUP EXPRESSION IN MALAWIAN PEDIATRIC MALARIA PATIENTS

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Sequestration of parasitised erythrocytes (pRBC) in the microcirculation of tissues is thought to be important in the pathogenesis of severe *falciparum* malaria. A major variant surface antigen, *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1), expressed on the surface of the pRBC mediates parasite cytoadhesion to vascular endothelium. PfEMP1 is encoded by the *var* multigene family that is sub-divided into three main groups, A, B and C, according to sequence similarities in coding and non-coding sequences. Using real time PCR, we compared abundance of the three main *var* groups utilising the resources of a clinicopathological study of fatal Malawian paediatric malaria patients. 20 patients were recruited and divided into cerebral malaria and parasitaemic control groups. The cerebral malaria group was sub-divided into two groups; circulating and sequestered parasites (CM1); circulating and sequestered parasites as well as perivascular abnormalities (CM2). *var* transcripts A and C were more abundant in the CM2 and the parasitaemic control group. However, a significantly different expression pattern was observed in the CM1 group, with *var* gene group B being more abundant than in the other two groups. This data indicates that perivascular pathogenesis in naturally infected children is associated with differential *var* gene expression in the body.

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C5 ACTIVATION MEDIATES ADVERSE COGNITIVE OUTCOMES IN OFFSPRING FOLLOWING *IN UTERO* EXPOSURE TO EXPERIMENTAL PLACENTAL MALARIA

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Each year approximately 125 million pregnancies are at risk of complications due to malaria infection. Despite the enormity of the problem and the serious implications of placental malaria (PM) on maternal and child health, little is known about the impact of PM on neonatal and infant cognitive development. Previous work has reported increased C5a in women with PM and has suggested that excessive activation of the host immune response, in particular of the complement system, may mediate adverse outcomes of PM. We hypothesized that *in utero* exposure to experimental PM will result in adverse cognitive and

neurological outcomes in offspring and that blockade of complement signaling will improve offspring outcomes. We used a murine model of PM that replicates several aspects of the human PM pathology to examine the impact of PM on the cognitive and neurological development of offspring as well as the role of C5 activation (C5a) using both genetic and receptor blockade strategies. BALB/c wild-type and C5a receptor deficient ($C5aR^{-/-}$) dams were infected at gestational day 13 with the rodent malaria parasite, *Plasmodium berghei* ANKA. In C5a-C5aR blockade studies, BALB/c dams were given anti-C5a antibody. Control animals were offspring brought to term by uninfected dams. Offspring were tested in a battery of behavioural tests to assess learning and memory, hyperactivity and affect. Neurological changes will be examined using MRI and brain tissue analyzed for levels of biogenic amines. We show that offspring of malaria-infected dams display an abnormal behavioural phenotype, including impairments in learning and memory in the novel object recognition test ($p < 0.0005$), and the y-maze test ($p < 0.05$), hyperactivity in the open field test ($p < 0.05$), and increased immobility in the tail suspension test ($p < 0.001$). Genetic and pharmacological blockade of C5a signalling confers protection against the behavioural phenotype ($p > 0.05$ across all tests). There was no significant difference in weight between groups up to eight weeks of age. Our results indicate that malaria-induced activation of the complement cascade contributes to the abnormal behavioural phenotype observed in offspring from malaria-infected dams independent of low birth weight. Moreover, strategies to block C5a signalling in malaria-infected dams rescue the behavioural impairments observed in offspring.

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CAN NEGATIVE EPISTASIS IN THE MALARIA PROTECTIVE EFFECTS OF SICKLE CELL TRAIT (HBAS) AND A+ - THALASSAEMIA BE EXPLAINED BY CYTOADHERENCE?

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The important role of malaria on human evolution is seen through the selection of high frequency polymorphisms that confer protection against malaria. Examples include the sickle cell trait (HbAS) and α+ - thalassaemia. The distribution of these polymorphisms overlaps in some malaria endemic regions where they can therefore be inherited in combination. Although their effect on protection against malaria when inherited individually is well described, little is known about their effect when inherited in combination. We have shown previously that the occurrence of HbAS in combination with α+ - thalassaemia results in a negative epistatic effect with a loss of protection against malaria afforded by each polymorphism individually. The exact mechanism through which this is mediated still remains unclear. We hypothesised that it could be explained by some of the important host-parasite interaction phenotypes associated with the pathogenesis of severe malaria including cytoadherence. Our results show that individually, HbAS and α+ - thalassaemia are associated with a reduced binding ability of *Plasmodium falciparum* infected erythrocytes to important recombinant endothelial receptors CD36 and ICAM1. Interestingly, this reduced binding ability is lost in erythrocytes containing both polymorphisms in a pattern very similar to that seen with the loss of protection from malaria in our epidemiological study. Preliminary results indicate that mechanisms other than altered PfEMP1 expression may be responsible for the changes in binding ability and we are studying the effect of HbAS and α+ - thalassaemia interaction on other important host-parasite related phenotypes including rosetting.

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PLASMODIUM KNOWLESI NORMOCYTE BINDING PROTEIN XA INVASION GENE HAPLOTYPES FROM HUMAN INFECTIONS IN MALAYSIAN BORNEO

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Parasitaemia is associated with disease severity in *Plasmodium knowlesi*, a zoonotic malaria, found in Southeast Asia. *P. knowlesi* has a 24-hour erythrocytic cycle and invades host red blood cells using multiple invasion ligands. The relative efficiency of invasion may be critical to the rate of increase in parasitaemia. Therefore a study was designed to test the hypothesis that *P. knowlesi* invasion gene haplotypes are associated with parasitaemia at presentation. The *P. knowlesi* normocyte binding protein xa (*Pknbpxa),* an invasion gene which is closely related to reticulocyte homologs of *P. falciparum* (Rh2a and Rh2b) was chosen for this work. In the first instance, the full-length *Pknbpxa* (8500bp) gene from *P. knowlesi* isolates from five patients was cloned and sequenced at high stringency. The five patients were recruited at geographically distinct locations and at different time intervals to identify polymorphic sites on the *Pknbpxa* gene. Preliminary analysis of these isolates show that there are 296 polymorphic sites within the gene. Full-length *Pknbpxa* DNA sequences will be presented highlighting polymorphic regions of the gene. A haplotyping method for screening *P. knowlesi* isolates from clinically well-characterized patients is being developed. *Pknbpxa* haplotypes will be analyzed for

associations with parasitaemia and other markers of disease severity in patients with *knowlesi* malaria recruited between January 2008 to April 2011 and the results presented.

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TRANSFER OF 4-HYDROXYNONENAL FROM PARASITIZED TO NON-PARASITIZED ERYTHROCYTES IN ROSETTES: ROLE IN SEVERE MALARIA ANEMIA

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Alpha+ thalassaemia occurs at high gene frequencies in malaria endemic regions. It is associated with low expression of complement receptor 1; reduced formation of rosettes and protection is specific for malaria-anemia. During parasite development, natural hemozoin catalyzes peroxidation of membrane lipids resulting in formation 4-hydroxynonenal (4-HNE) that forms adducts with proteins of the red blood cell membrane resulting in modification of the cytoskeleton responsible for cell deformability which might cause the increased removal of non parasitized RBCs in the spleen as seen in malaria anemia. 4-HNE also has the ability to diffuse to surrounding cells. In this study, therefore, we hypothesized that in rosettes 4-HNE may diffuse from parasitized to non-parasitized RBCs, damage non-parasitized RBCs and induce their phagocytic removal, providing a mechanistic explanation for the association of rosetting with severe malaria anemia and protection afforded by alpha+ thalassemia. Cultures of varO variant of *Plasmodium falciparum* experiments showed transfer of 4-HNE to non-parasitized RBCs within rosettes. Ex vivo analysis of samples from malaria patients (N=40) showed an increase in the proportion of non-parasitized RBCs positive for 4-HNE adducts with increasing rosette frequency. Anemic children had a significantly higher proportion of non-parasitized RBCs positive for 4-HNE adducts than non-anemic children (P=0.0037). However, neither rosette frequency nor proportion of non-parasitized RBCs positive for 4-HNE-adducts differed by thalassemia genotype. 4-HNE adducts are present in infected and uninfected RBCs in culture. There is a dependency of 4-HNE positivity of uninfected RBC with ability to form rosettes. Increased 4-HNE modification of uninfected RBC may explain the correlation between rosetting and malaria anaemia. No correlation was found between thalassaemia genotype and rosetting or HNE modification.

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SEQUESTRATION ASSOCIATED LOSS OF PROTEIN C RECEPTORS LINKS COAGULATION, INFLAMMATION AND ENDOTHELIAL PERMEABILITY IN CEREBRAL MALARIA

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Studies in comatose Thai adults implicate coagulation abnormalities in cerebral malaria (CM). In African children, this relationship is supported by vascular hemorrhagic lesions found in the retina in life and in the brain post mortem. We hypothesised that these lesions indicate intravascular coagulation caused by an imbalance in the thrombin and protein C pathways. Immunohistochemistry (IHC) on post mortem brain tissue from 3 Malawian children with fatal CM showed that the characteristic vascular lesions contain fibrin thrombi, implying a state of thrombin dysregulation. Thrombin-antithrombin complexes in 71 Malawian children with CM were significantly raised when compared with healthy controls (n=19, p=<0.0001), mild febrile illness (n=30, p=<0.05) or uncomplicated malaria (n=30, p=<0.001). To explore the possibility that loss of the anticoagulant receptors thrombomodulin (TM) and endothelial protein c receptor (EPCR) explains this increased thrombin generation and fibrin deposition associated with CM, we performed IHC on post mortem CM brain, gut and subcutaneous tissue. This showed a unique pattern of TM and EPCR distribution with normal expression in healthy vessels but absence in vessels containing malaria parasite infected erythrocytes (IE). To confirm these findings pre-mortem, we developed a novel flow cytometry method to assess ICAM-1, TM and EPCR expression on the microvessels of subcutaneous fat, a tissue that sequesters IE. This revealed a marked decrease in endothelial TM (p=<0.005) and EPCR (p=<0.005) but an increase in ICAM-1 (p=<0.0001) compared with controls. This dysregulation in the thrombin and protein C pathways, with focal loss of the major anticoagulant and endothelial protective receptors at sites of IE sequestration, provides new insight into CM pathogenesis. It provides a link between key pathogenic features of the disease; coagulation, inflammation and vessel permeability. Furthermore, because TM and EPCR are constitutively expressed at lower levels in cerebral vessels, it may explain the vulnerability of the brain in this condition.

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A BIBLIOMETRICS ANALYSIS MALARIA RESEARCH

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Bibliometric analyses indicate trends and patterns within scientific disciplines, national and international strengths and biases in areas of research. In view of the importance of malaria research in the field of human health, it is essential to shed light on research activities carried out around the world. Since publications are one of the major outputs of any research activity which can be quantified, the objective is to map the international and Brazilian research production, skills and competence along with the key research topics/themes of malaria research in the world and in Brazil. We also identify the pattern of malaria research funding, which should provide subsidies to improve public policies. This project is part of the the National Institute of Science and Technology for Innovation in Neglected Diseases (INCT-IDN, http://www.cdts.fiocruz.br/AnnualActivityReport/), based at the Oswaldo Cruz Foundation in Brazil. References on the subject obtained from the Science Citation Index (SCI), PubMed Medline and Scopus databases for the period 1997-2010 are analyzed. A total of 78,742 articles were identified for each database respectively: SCI (41,022), Medline (35,539) and Scopus (2,181). The results show that the research takes place mainly in Europe and North America, not the peripheral countries who are directly affected by the disease. In accordance to other publications of bibliometric analysis of malaria and tropical medicine, the main bias with all these databases is that they are dominated by North American and European publications, and by default, authors. A further bias is their inaccessibility to authors from developing countries. Internationally, the most substantial funding for malaria research came from the National Institutes of Health, and World Health Organization. In Brazil, these were The National Council for Scientific and Technological Development and the State of São Paulo Research Foundation. These data are preliminary and we intend to extend the searches to other databases and also use network analysis to detect collaborative research groups or communities in this area.

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ANTI-GLYCOPHORIN A AND B ANTIBODIES INHIBIT ERYTHROCYTIC INVASION OF *BABESIA DIVERGENS*

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Babesiosis is an emerging tick borne zoonotic disease caused by intraerythrocytic parasites of the genus *Babesia*. *B. microti* and *B.*

divergens have been recognized as important infectious agents in humans and as a threat for transfusion medicine. Although the incidence of *B. divergens* is not as high as *B. microti*, *B. divergens* causes more severe disease. *B. divergens* is the only human babesia parasites that can be propagated in human red blood cells (RBC) *in vitro* with exceptionally high parasitemia (up to 80%). Because of the parallels in the invasion patterns of *Plasmodium* and *Babesia* into human erythrocytes, we are interested in building *B. divergens* into a parasite model to study malarial RBC invasion. In contrast to *Plasmodium*, little is known about the *Babesia*-host cell interaction process. *B. divergens* specifically invades RBCs. Previous work in our lab has identified the Glycophorins A (GPA) and B (GPB) as host receptors for parasite invasion. In this work, we evaluated the participation of GPA and GPB specific domains in RBC invasion by *B. divergens*. Inhibition-of-invasion assays were carried out in the presence of specific monoclonal antibodies directed against various GPA and GPB determinants. We have identified 2 MAbs and 2 Fab fragments that caused a significant decrease in invasion efficiency when compared to the control MAbs. These results represent an important advance toward the identification of binding domains on GPA and GPB involved in the receptor-ligand interactions that facilitate the entry of *B. divergens* merozoite into the human erythrocyte.

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CD36 RECRUITMENT AND ACTIN CYTOSKELETAL REARRANGEMENT VIA P130CAS ENHANCES AVIDITY OF *PLASMODIUM FALCIPARUM* ADHESION ON HUMAN MICROVASCULAR ENDOTHELIUM

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The adhesion of *Plasmodium falciparum*-infected erythrocytes (IRBC) to microvascular endothelium is critical in the pathogenesis of severe malaria. In this study, we used atomic force microscopy to analyze the adhesive force between IRBC and human dermal microvascular endothelial cells (HDMEC). A single live IRBC, attached to the end of the cantilever, served as a functionalized probe that monitored the IRBC-endothelial cell interaction in real time. Our results show that the initial IRBC-HDMEC interaction generated a mean adhesion force of 166.7 ± 4.2 pN pN from the formation of either single or multiple bonds. The adhesion force was reduced by an anti-CD36 but not an anti-ICAM-1 antibody. Interestingly, the adhesion force increased with time as the IRBC was left in contact with the endothelium, so that by 300 seconds the force of adhesion had increased to 559.3 ± 45.5 pN. The time-dependent increase in the strength of adhesion was mediated by CD36, Src family kinases, the adaptor protein p130CAS, and actin cytoskeletal rearrangement in a calcium-dependent manner. The end result was both an increase in the affinity of binding between IRBC and HDMEC that was alkaline phosphatase dependent, and an increase in the number of ligand-receptor pairs through cytoskeletal rearrangement. These findings were supported by fluorescence microscopy imaging that showed recruitment of CD36 and actin in response to ligation of CD36 by IRBC, anti-CD36 or parasite peptide coated beads. Functionally, the increase in adhesion force enabled IRBC to remain adherent in shear stresses of up to 15 dynes/cm2 in a flow chamber assay, an ability that was significantly reduced on HDMEC in which p130CAS expression was knocked down by siRNA. Collectively, the data suggest a novel mechanism by which IRBC adhesion to CD36 activates a signaling pathway that leads to changes in the membrane localization of CD36, actin recruitment and increased binding avidity between IRBC and HDMEC. These results provide new insight into the complex regulation of cytoadherence by *P. falciparum* that could be exploited in the development of novel therapeutic interventions.

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NOVEL MODEL OF SEVERE MALARIAL ANEMIA USING SEQUENTIAL *PLASMODIUM CHABAUDI* AND *P. BERGHEI* INFECTION

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Lack of an adequate animal model of *Plasmodium falciparum* severe malarial anemia (SMA) has hampered the understanding of this highly lethal condition. Therefore, we set out to develop a model in mice that reflected key characteristics of SMA in humans such as relatively low parasitemia and the requirement of pre-immunity. We found that C57BL/6 mice infected with *P. berghei* after recovery from *P. chabaudi* (Pch-Pb) developed an initial 9-10-day phase of relatively low parasitemia and severe anemia followed by a second phase of hyperparasitemia, more profound anemia, reticulocytosis, and death 14-21 days after infection. We studied the first phase of this infection as a model of SMA. Pch-Pb animals had more intense splenic hematopoiesis, higher IL-10/TNF-α and IL-12/IFN-γ ratios, and higher antibody levels against *P. berghei* and *P. chabaudi* antigens, than *P. berghei*-infected (Pb) or *P. chabaudi*-recovered (Pch-sham) animals. Early treatment with chloroquine or artesunate did

not prevent the anemia, suggesting that the bulk of red cell destruction was not due to the parasite. Red cells from Pch-Pb animals had increased surface IgG and C3 by flow cytometry. However, $C3^{-/-}$ mice still developed anemia. Cell tracking of *ex vivo* and *in vivo* labeled red cells and analysis of tissue sections by H&E and immunofluorescent microscopy demonstrated that red cells from Pch-Pb animals were removed at an accelerated rate in the liver by erythrophagocytosis. We conclude that this model is practical and reproducible. Its similarities with *P. falciparum* SMA in humans makes it an appealing system to study the pathogenesis of this condition and explore potential immunomodulatory interventions.

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A SYSTEMS IMMUNOLOGY APPROACH TO UNDERSTANDING THE ACQUISITION AND LOSS OF IMMUNITY TO MALARIA A BETTER

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A better understanding of how immunity to malaria is acquired and lost will be increasingly important as efforts to eliminate malaria proceed, not only to inform novel malaria vaccine strategies, but to understand how interventions such as mass drug administration might influence malaria susceptibility in populations at different stages of malaria control and elimination. In an area of Mali that experiences an intense six-month malaria season we conducted a longitudinal study in which flow cytometry, multiplex cytokine analysis, and genome-wide transcription profiling were used to analyze the impact of acute *Plasmodium falciparum* (Pf) infection on the human immune system Peripheral blood mononuclear cells (PBMCs) were collected from Pf-uninfected children at the end of the dry season, and again after the first malaria episode of the year. Genome-wide expression analysis identified 581 transcripts with a >2 fold change in expression from before to after the malaria episode. Both the innate and adaptive branches of the immune system were affected as evidenced by alterations in the expression of cytokines including IFNγ, TNFα, TGFβ, IL-1 and IL-4, as well as components of Toll-like receptor (TLR), B cell receptor (BCR) and T cell receptor (TCR) signalling pathways. Taken together, preliminary analyses are consistent with the hypothesis that the relatively rapid acquisition and loss of strain-transcendent immunity to severe malaria is due in part to down regulation of pro-inflammatory pathways, a hypothesis that we are testing further in a larger prospective cohort study in Mali and by several *in vitro* experimental approaches. This study highlights how advances in genome-based technology can be applied to longitudinal studies in which malaria exposure is clearly defined to gain insight into how malaria immunity is acquired and lost.

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REGULATION OF AKT/GSK-3 AND NEUROPROTECTION WITH CEREBRAL MALARIA

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Cerebral Malaria (CM) is the most severe neurological complication of *Plasmodium falciparum* infection, resulting in an encephalopathy. Previously, we demonstrated that infection of C57BL/6 mice with *P. berghei* ANKA (PbA) was associated with a vasculopathy which results in a reduction of cerebral blood flow, neuronal dysfunction and axonal impairment. These were associated with neuro-cognitive and motor deficits in CM mice both during acute infection and after successful anti-parasitic treatment. The mechanisms that underlie the lingering effects of the neuronal damage and cognitive deficits in CM remain largely unknown. Here we demonstrate that acute CM results in significantly abnormal phosphorylation of tau protein. This aberrant tau phosphorylation is associated with a significant reduction in the activation of Akt in the brains of mice with CM leading to a significant decrease in Akt inhibition of glycogen synthase kinase (GSK3-β). We demonstrate that regulation of GSK3-beta is neuroprotective in mice with CM. Treatment with lithium chloride, a compound that regulates GSK3- β activity, ameliorates the neuro-cognitive and motor deficits in PbA-infected mice after eradication of the parasite. This indicates that regulation of GSK3- β may reduce neuronal degeneration and have neuroprotective effects in CM. Our data present GSK-3 as a potential therapeutic target which might be used as adjunctive therapy directed at the reduction of neurological dysfunction in children with CM.

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SIMULTANEOUS QUANTIFICATION OF ASEXUAL AND SEXUAL STAGES DURING MALARIA INFECTION

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Determining the prevalence of *Plasmodium falciparum* gametocyte carriers in the population is important because peripheral gametocytemia is a major determinant of transmission in this devastating disease. Detection and quantification of gametocytes in the blood of malaria-infected patients is generally based on microscopy, which is tedious and lacks sensitivity. Newer transcript-based methods have succeeded in quantifying these stages, but there is not a standardized approach for quantifying asexual and sexual stages in the same sample with adequate sensitivity and detailed stage resolution. We have thus developed a model for predicting the sample proportions of two asexual (ring and schizont) and three sexual (early, intermediate and mature) stages based on transcript levels. Using published expression data from across the *P. falciparum* intraerythrocytic infection cycle, we identified five genes with peak expression occurring during one of the aforementioned stages and one gene with constitutive expression across all stages. This provided us with a set of six "sentinel" genes, each of which was informative about particular stage(s). We deliberately selected intron-containing genes so that primers could be designed across exon-exon junctions. This strategy increases the sensitivity of the process by preventing amplification of residual genomic DNA. We performed qRT-PCR for the sentinel genes at several time points during *in vitro* asexual and sexual development time course experiments. Using the qRT-PCR data in combination with stage-specific microscopy data for each time point, we developed a constrained linear regression model to predict the stage composition for an unknown sample. Testing and validation of model accuracy was done using *in vitro* samples with known stage composition. Finally, we applied our qRT-PCR assay and predictive model to a set of patient blood samples and obtained estimates for the percent of parasites in each life cycle stage category. We believe this system may be suitable for malaria control and elimination activities, where the identification and detailed resolution of gametocyte life cycle stages is required.

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EXPLORING PROVIDER AND COMMUNITY RESPONSES TO THE NEW MALARIA DIAGNOSTIC AND TREATMENT REGIME IN SOLOMON ISLANDS

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Improvements in availability and accessibility of artemisinin-based combination therapy (ACT) for malaria treatment and the emergence of multi-drug-resistant parasites have prompted many countries to adopt ACT as the first-line drug. In 2009, Solomon Islands (SI) likewise implemented new national treatment guidelines for malaria. The ACT, artemether-lumefantrine is now the primary pharmacotherapy in SI for *Plasmodium falciparum* malaria, *Plasmodium vivax* malaria and mixed infections. Targeted treatment is also recommended in the new treatment regime through maintenance of quality microscopy services and the introduction of Rapid Diagnostic Tests (RDTs). Ascertaining the factors that influence community and provider acceptance of and adherence to the new treatment regime will be vital to improving the effectiveness of this intervention and reducing the risk of development of drug resistance. To understand community and prescriber perceptions and acceptability of the new diagnostic and treatment regime, 12 focus group discussions and 12 key informant interviews were carried out in rural and urban villages of Malaita Province, Solomon Islands, four months subsequent to roll out of these interventions. Lack of access to microscopy or distrust in the accuracy of diagnostic tools were reported by some participants as reasons for the ongoing practice of presumptive treatment of malaria. Lack of confidence in RDT accuracy negatively impacted its acceptability. Artemether-lumefantrine had good acceptability among most participants; however, some rural participants questioned its effectiveness due to lack of side effects and the larger quantity of tablets required to be taken. Storing of left over medication for subsequent fever episodes was reported as common. To address these issues, further training and supportive supervision of healthcare workers will be essential, as will the engagement of influential community members in health promotion activities to improve acceptability of RDTs and adherence to the new treatment regime. Exploring the extent of these issues beyond the study population must be a priority for malaria programme managers. Practices such as presumptive treatment and the taking of sub-curative doses are of considerable concern for both the health of individuals and the increased risk it poses to the development of parasite resistance to this important first-line treatment against malaria.

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COSTING A LARGE-SCALE IMPLEMENTATION OF INTERMITTENT PREVENTIVE TREATMENT OF MALARIA IN CHILDREN DELIVERED THROUGH COMMUNITY HEALTH WORKERS IN SENEGAL

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Intermittent Preventive Treatment in children (IPTc) is a new strategy for malaria control in areas where transmission is strongly seasonal, shown to be highly effective in clinical trials. In Senegal, a pilot implementation of IPTc was conducted by four district health teams from 2008 to 2010 in order to evaluate the feasibility of delivering IPTc, its safety and effectiveness, when administered on a large scale to rural populations using community health workers (CHWs). In 2010, the intervention was delivered by 46 health-posts to a rural population of 175,000 children under 10 years of age in 1097 villages, and detailed information on costs was collected from each health facility in order to estimate the financial and economic costs of delivery. Delivery was coordinated by the head nurse in each health-post who assigned CHWs to a circuit of villages to visit over a 5-day period in September, October and November, to deliver IPTc house to house to all children 3-120 months of age. Tools were developed to collect data on costs and resource use at four levels: the project, the district, the health post, and the CHW. Data was collected from both "top-down", and "bottom-up" (using facility-based costs and extensive interviews on resource use). Data were collected from all 46 health-posts after each round of administration. The study takes a provider perspective with a focus on costs of implementation at the district level. Each health-post employed from 4-68 CHWs and delivery each month took from 2-5 days. High coverage was achieved with about 90% of eligible children treated each month. When the financial cost of delivery was estimated, it cost $233,714 to administer IPTc to a population of 175,000 children under 10 years of age at a cost of $0.50 per course. High coverage of IPTc can be achieved at moderate cost. Each year CHWs may visit households a number of times for distribution of Vitamin A, bednets, mass vaccination and other programmes. Opportunities therefore exist for economies of scope by combining IPTc with delivery of other interventions.

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ANTIMALARIAL DRUG UTILIZATION IN A CHILDREN HOSPITAL

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Although drugs have greatly transformed the practice of medicine in recent years, inappropriate drug use can lead to increased mortality and morbidity rates. National and international health organizations therefore employ policy changes in order to regulate and optimize the benefits of drug use. This study investigated drug utilization in a children's hospital in Ibadan, Southwest Nigeria. In particular it investigated the impact of a national antimalarial drug policy change on prescribing patterns. Patients' case note data on age, sex, diagnoses and drug therapies during a single hospital visit were reviewed and assessed using selected WHO drug use indicators. One out of ten prescriptions written in the first four months of 2004, 2005 and 2010 were studied retrospectively. Data analysis was done using SPSS 14 with confidence limits set at 95%. Percentage of prescriptions including artemisinin based combination therapies (ACTs) though negligible in 2004 increased to more than 60% in 2010. There was an attendant reduction in the proportion of prescriptions written in generic names as many ACTs were prescribed using brand names. In addition, compliance with the policy was not significant until nine months after the national adoption of the policy.This study provides information on trends of antimalarial drug use and responsiveness to antimalarial treatment policy. The study confirms that drug policy changes are not without difficulties and require sustained monitoring to succeed.

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IN VITRO ACTIVITY OF FERROQUINE, VERSUS CHLOROQUINE, AGAINST WESTERN KENYA *PLASMODIUM FALCIPARUM* FIELD ISOLATES AS DETERMINED BY A SYBR GREEN I ASSAY

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Ferroquine (FQ), a 4-aminoquinoline analogue of chloroquine (CQ), is being developed for treating CQ resistant and CQ sensitive *Plasmodium falciparum* malaria. Growing *in vitro* drug sensitivity data support these indications, and that FQ may be more potent than CQ. Continued *in vitro* testing, especially against CQ resistant *P. falciparum* field isolates, may be useful in understanding of FQ's potential. In 146 *P. falciparum* field isolates collected in western Kenya, most processed immediate *ex vivo* (IEV), we measured 50% inhibitory concentrations (IC_{50}; nM) of CQ and FQ by a SYBR Green I *in vitro* assay. Laboratory reference clones included D6 (CQ "resistant") and W2 (CQ "resistant"). Field isolates were assessed for PfCRT K76T mutation, *Pfmdr1* copy number and *Pfmdr1* single nucleotide polymorphisms (SNPs) at 4 codons by real time PCR. Geometric mean IC_{50}s for FQ were lower than CQ for *P. falciparum* field isolates ($p = 0.005$) and the CQ resistant clone W2 ($p < 0.001$). pfcrt K76 mutations, detected in > 80% of isolates, conferred higher IC_{50}s for CQ, and modestly lower IC_{50}s for FQ. For *Pfmdr1*, mean copy number was 1, with SNPs common at codon 86. In conclusion, *in vitro*, FQ is more potent than CQ against CQ-resistant *P. falciparum* field isolates, and the CQ-resistant clone W2, with seemingly little or no effect from pfcrt K76T mutations. This bodes well for the clinical use of ferroquine.

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FERROUS IRON-TARGETED DRUG DELIVERY IN ANTIMALARIAL CHEMOTHERAPY

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Artemisinin combination therapy is the current standard of care in treating uncomplicated malaria. While the precise mechanism of artemisinin action is still debated, the importance of an initial reductive activation by ferrous iron is broadly accepted in the field. We have been exploring novel means to deliver multiple drug activities to the malaria parasite in a ferrous iron-dependent fashion. This new approach has the potential improve drug efficacy and safety and to reduce the potential for resistance, particularly in the context of prophylaxis. Here we will describe prototypical ferrous-iron targeted prodrugs that deliver a pan protease inhibitor only after an initial 1,2,4-trioxolane (artemisinin-like) activity has been conferred. Activity-based probes were used to confirm ferrous iron-dependent drug delivery in cultured parasites. *Plasmodium berghei* infected mice were cured when treated for three days (40 mg/kg/day, ip) with the prodrug, treatment starting ten days post-inoculation. This delivery of a protease inhibitor in prodrug form was both more efficacious and much less toxic than direct administration of the protease inhibitor alone at a comparable dose.

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SIMULTANEOUS ANALYSIS OF PRIMAQUINE AND ITS METABOLITE CARBOXYPRIMAQUINE BY LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY

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Primaquine (PMQ) is the only tissue schizonticide that is widely used in the treatment of *Plasmodium vivax* malaria. However, a variety of dosage regimens are reported and the pharmacokinetics of PMQ and its principal active metabolite, carboxyprimaquine (CPMQ), have not been clearly established in some patient groups, including children. In most pharmacokinetic studies, PMQ and CPMQ have been extracted and analysed separately by HPLC methods, because PMQ is a base and CPMQ is an acid. There are few reported LC-MS methods that are reproducible and applicable in clinical trial assays. We have developed a simple, robust method to simultaneously extract and analyze both PMQ and CPMQ from plasma. Solid phase extraction was used for sample preparation (Waters Oasis HLB cartridges) with 8-aminoquinoline as the internal standard. Analyses were performed using a Shimadzu 2020 LC-MS with selected ion monitoring under electrospray ionisation mode (m/z for quantification was 260, 275 and 145 for PMQ, CPMQ and 8-aminoquinoline respectively). Separation was performed isocratically in water and methanol (20:80) containing 0.1% formic acid, using a Phenomenex Luna C18 column. Retention times for PMQ, CPMQ and 8-aminoquinoline were 3.4, 8.6 and 5.9 min respectively. Standard curves were linear across the concentration range 2-1000 µg/L. Analysis of samples containing three different PQ and CPMQ concentrations (5, 50 and 200 µg/L) spiked into five separate plasma samples were used to determine matrix effects (ion suppression/enhancement), absolute recovery, and process efficiency, all of which were within acceptable analytical ranges. The assay intra-day and inter-day relative standard deviations were <10% at 5, 50, 200, 500 and 1000 µg/L. LLOQ for PMQ and CPMQ were 1 µg/L and 2 µg/L respectively. Plasma concentration PMQ and CPMQ profiles for a representative patient were consistent with previously-reported data. A simple and reliable LC-MS method was developed, validated and successfully applied to determine PMQ and CPMQ plasma concentrations in patients treated with primaquine.

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POTENTIAL FOR CLINICAL IMPACT FROM THE NON-HEMOLYTIC 8-AMINOQUINOLINE CONSORTIUM

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Primaquine (PQ) is an 8-aminoquinoline (8AQ) critical for malaria elimination campaigns, although its widespread use is limited by hemolysis in G6PD-deficient (G6PDd) people and poor adherence. Tafenoquine (TQ), an analog with a two-week half life, is currently in Phase II/III clinical trials for *Plasmodium vivax* radical cure. In 2008, an expert committee reviewed the current state of knowledge and made key recommendations. All available safety and efficacy data from 1800+ 8AQ from the WRAIR chemical inventory system, the literature, and the 40 given to humans are available in a website database. To understand and overcome hemolytic risk of this drug class, one *in vitro*, two mouse and a Rhesus model of G6PDd have recently been qualified. These models are allowing us to implement a systematic plan to understand the mechanisms of hemolysis, the impact of drug combinations, and identification of non-hemolytic 8AQs. The 12 drugs documented to be hemolytic in G6PDd humans and 12 non-hemolytic analogs were used to make a model that correctly predicted an 8AQ efficacious in monkeys to be non-hemolytic. Both TQ and NPC1161B appear to have a clearly improved therapeutic index over PQ. In the human literature, chloroquine and quinine potentiate activity against hypnozoites, while quinine decreases methemoglobin, the mechanism of which is now being understood through key metabolism experiments. Initial studies in mice have suggested that a pan cytochrome p450 inhibitor blocks efficacy of 8AQ, but not hemolytic toxicity, providing initial evidence that efficacy can be separated from toxicity. Drugs in human use will be systematically evaluated in combination and with new approaches to determine if an improved therapeutic index is achievable in the newly qualified G6PDd models and efficacy models in the same animal strains/species. Combinations will target mechanisms to reduce hemolysis (e.g. antioxidants, metabolism inhibitors) or potentiate efficacy (e.g. chloroquine). Promising approaches will be pushed to human testing for definitive documentation of proof of concept.

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IN VITRO PLASMODIUM FALCIPARUM KILLING RATES CAN DIFFERENTIATE ANTIMALARIAL MODE OF ACTION

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Malaria is a major public health and economical issue affecting the world's most disadvantaged populations. Current treatments are compromised by the advance of resistance even to the newest antimalarial class, highlighting our constant need for new and efficacious drugs. Ideally new drugs should be fast-acting compounds in order to maximize their therapeutic efficacy and minimize their potential to induce resistance. Current assays to assess antimalarial potency of compounds are based on parasite metabolism measures, however these methods are not adequate to evaluate effects over parasite viability: metabolically inactive parasites can be viable because drug effect can be fully reversible or parasites committed to death might still appear metabolically active as is the case for most of antibacterials with antimalarial efficacy. We present an *in vitro* methodology to address these issues, that assess viability of drug-treated parasites over time. This method uses limiting-dilution technique to quantify the amount of viable parasites, and provides an *in vitro* pharmacodynamic profile for each antimalarial drug. Relevant PD parameters can be determined by this method such as - *in vitro* parasite reduction rate (PRR) representing the fractional reduction of initial parasites load per asexual life cycle or the lag phase that reflects the time needed to observe full killing effects of a drug. Furthermore, by testing increasing concentrations of the drug, conditions for the maximum killing rate can be determined. This can be useful to select the optimal doses for efficacy in clinical trials. Moreover, *in vitro* PRR profiles appear to be directly related to the antimalarial drug mode-of-action. Drugs acting on the same target or pathway display similar profiles. This observation suggests that PRR profiles can be used to distinguish different antimalarial mode-of-action and potentially identify the drugs with the fastest killing rate.

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"THEY JUST TOLD ME TO GO BUY DRUGS FROM THE SHOP." HOW THE 2010 ACT STOCK OUTS IN TANZANIA AFFECTED MALARIA CASE MANAGEMENT AND CARE-SEEKING BEHAVIORS IN MTWARA REGION

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We present qualitative data from two communities in Mtwara Region to examine how stock outs of Artemether-Lumefantrine (ALu) during February - June 2010 affected malaria case management and care-seeking behaviors. We conducted qualitative interviews to examine provider and community experiences with malaria diagnosis and treatment including 21 provider in-depth interviewees (IDI), 8 community focus groups (FGD), and 31 illness narrative interviewees (INI) who had experienced a recent malaria episode. Discussions were held with regional and district authorities to document malaria-related strategies and challenges. Data were collected twice to capture seasonal differences. Interview transcripts were entered into NVivo8 for content-analysis. Although all INI who sought malaria treatment reported using some type of antimalarial drug (AM), INI seeking care during the stock out period were half as likely to be treated with ALu compared to INI pre-stock out. Stock outs often resulted in patients having to travel back and forth between health facilities (HF) and drug shops (DS) in search of prescriptions and drugs. Although some health worker IDI said they provided patients with prescriptions for DS referrals, several INI reported being left on their own to figure out where to go and what AM to buy. Others noted they avoided particular HF altogether either because of their own or others' prior experiences of being told there were no drugs. Economic consequences of ALu stock outs also emerged. Both FGD and INI participants complained about having to pay HF registration fees only to be told there were no drugs; some INI resorted to buying AM half doses due to limited funds. District authorities adopted several measures to address the stock out including rationing of ALu to under-fives, moving ALu stocks between HFs, and treatment with available AM, although they did note their concern with the overuse of sulfadoxine-prymethamine and quinine. The region received 480 doses of Duocotexin from the Medical Stores Department to use as an alternative ACT treatment but it eventually

expired. HF stock outs of ALu in 2010 had negative effects on timely access to effective antimalarial treatments. Attention to improving timely acquisition and distribution of ACTs is of utmost importance.

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IN VITRO METABOLISM OF PIPERAQUINE IS PRIMARILY MEDIATED BY CYP3A4

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Piperaquine (PQ) is a quinoline antimalarial that has been recently added as a first-line treatment option for uncomplicated *Plasmodium falciparum* malaria by the World Health Organization. The primary objective of this investigation was to determine the major metabolic pathway(s) of PQ *in vitro*. A reliable, validated tandem mass spectrometry method was developed to quantify PQ. Concentrations of PQ were measured over time after incubation with both human liver microsomes (HLMs) and expressed cytochrome P450 enzymes (P450s). In pooled HLMs, incubations with an initial PQ concentration of 0.3 μM resulted in a 34.8 + 4.9% loss of substrate over 60 min, corresponding to a turnover rate of 0.009 min-1 (r2 = 0.9223). Miconazole, at non-specific P450 inhibitory concentrations, resulted in almost complete inhibition of PQ metabolism in HLMs. The greatest inhibition was demonstrated with selective CYP3A4 (100%) and CYP2C8 (66%) inhibitors. Using a mixture of recombinant P450 enzymes, turnover for PQ metabolism was estimated as 0.0099min-1; recombinant CYP3A4 had a higher metabolic rate (0.017 min-1) than recombinant CYP2C8 ($p < 0.0001$). Inhibition of CYP3A4-mediated PQ loss was greatest using the selective inhibitor ketoconazole (9.1 + 3.5% loss with ketoconazole vs 60.7 + 5.9% with no inhibitor, $p < 0.0001$). The extent of inhibition of *in vitro* metabolism with ketoconazole (83%) denotes that PQ appears to be primarily catalyzed by CYP3A4.

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DETERMINANTS OF PROMPT ANTIMALARIAL TREATMENT OF FEVER IN CHILDREN UNDER FIVE: EVIDENCE FOR TARGETED COMMUNICATIONS IN FIVE AFRICAN COUNTRIES

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In malaria endemic settings, decisions made by children's caregivers at the onset of fever are critical. While increasing access to artemisinin combination therapy (ACT) is important for improving effective fever treatment, a number of demand-side factors likely influence treatment outcomes. Identifying determinants of treatment-seeking behavior can inform demand creation activities aimed at achieving optimal uptake of ACTs. This study uses a behavior change framework to guide examination of opportunity, ability and motivation factors theorized to influence prompt antimalarial treatment for fever in children under five. Formative in-depth interviews and focus group discussions informed development of quantitative scaled constructs. Nationally-representative surveys were conducted in DRC, Madagascar, Nigeria, Uganda and Zambia during 2008-2010 as part of the ACTwatch research program. Logistic regression was used to build country-specific models predicting prompt antimalarial treatment of fever. Results show that each country context is unique with respect to determinants of behavior and the gap between current and ideal levels of those determinants among caregivers. Implications for program design are discussed. Cross-country trends include significance of opportunity (perceived antimalarial availability) and motivation factors (beliefs favorable to modern medicine and positive outcome expectations for use of antimalarials). Ability factors that were tested (malaria knowledge and forms of social support) are not typically significant behavioral determinants. Relative household wealth is not associated with prompt antimalarial treatment, with the exception of a significant disparity in Nigeria. As effective antimalarials become more affordable and available, locally-relevant evidence-based communications will play an important role in ensuring that caregivers promptly seek and acquire these treatments. Practical methods for investigating behavior can provide necessary evidence to create targeted communications.

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TEN YEARS EXPERIENCE WITH COARTEM: A PATIENT-CENTRIC APPROACH TO FIGHTING MALARIA

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We review the experience made with Coartem (Artemether-Lumefantrine), the gold standard artemisinin-based combination therapy (ACT) for malaria that has been deployed to endemic countries for the last 10 years, delivering over 400 million treatments. Over the years, our focus shifted from providing a quality medicine in public/private partnership with WHO to a holistic, 'patient-centric' approach, focusing on educating caregivers and patients to ensure, timely treatment and adherence to full course of medication, involving multiple partnerships. To meet the specific needs of children, a dispersible formulation was developed jointly with Medicines for Malaria Venture (MMV). Coartem Dispersible tablets meet the specific needs of children as they can be given dispersed in a small amount of liquid and are sweetened to mask the bitter taste, which is typical of most antimalarials. More recently, we are evaluating novel approaches that may be of use in malaria elimination strategies. A study assessing the utility of Coartem in mass screening and targeted treatment for malaria in entire village populations, including carriers of the malaria parasite that are asymptomatic has been undertaken in an effort to reduce parasite transmission. New strategies to expand access to ACTs have been implemented: the Affordable Medicines Facility - malaria (AMFm) initiative, where funds from donors will be used as subsidies to lower the price of ACTs at retail outlets, and the SMS for Life initiative, part of the Roll Back Malaria (RBM) program, a tool for supply chain management based on electronic mapping technology and short text messages sent via mobile phones. These initiatives that maintain and further evolve a patient-centric approach, and go beyond a mere deployment of drugs are essential for achieving a sustained health benefit in developing countries. Sharing these learnings with relevant stakeholders may allow developing strategies that achieve similar results also for other diseases, worldwide.

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CYCLOPROPYL CARBOXAMIDES A NEW ANTIMALARIAL CHEMICAL CLASS WITH *IN VIVO* EFFICACY

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Discovery of new classes of antimalarial drugs has become an urgent task to counteract the increasingly problem of drug resistance. Screening directly for compounds able to inhibit parasite growth *in vitro* is one of the main approaches the malaria research community is now pursuing for the identification of novel antimalarial drug leads. Very recently, thousands of compounds with potent activity against the parasite *Plasmodium falciparum* have been identified and information about their molecular descriptors, antiplasmodial potency and cytotoxicity is publicly available. Now the challenge is how to identify the most promising chemotypes for further development and how best to progress these compounds through a lead optimization program to generate antimalarial drug candidates. We report here the first chemical series to be characterized from one of those screens, a completely novel chemical class designated with the generic name of cyclopropylcarboxamides and never before described to have antimalarial or other pharmacological activities. Cyclopropylcarboxamides are potent inhibitors of drug sensitive and resistant strains of *P. falciparum in vitro*, and show *in vivo* oral efficacy in malaria mouse models. In this work we describe the biological characterization of this chemical family,

showing that inhibition of their still unknown target has very favorable pharmacological consequences but the compounds themselves seem to select for resistance at high frequency.

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NEUROLOGICAL SEQUELAE OF CEREBRAL MALARIA IN CHILDREN

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Out of 604 Rwandan children admitted with *falciparum* malaria to Ndera hospital hospital between August and December, 2009, 308 had cerebral malaria and 203 were severely anaemic (haemoglobin less than 60 g/l). 14% of those with cerebral malaria died, as did 7.8% of those with severe anaemia. 32 (12%) of children surviving cerebral malaria had residual neurological deficit. 69 other children were admitted with clinical features strongly suggestive of cerebral malaria but with negative blood films; 16 of these died and 3 had residual neurological deficits. The commonest sequelae of cerebral malaria were hemiplegia (23 cases), cortical blindness (11), aphasia (9), and ataxia (6). Factors predisposing to sequelae included prolonged coma, protracted convulsions, severe anaemia, and a biphasic clinical course characterised by recovery of consciousness followed by recurrent convulsions and coma. At follow up 1-6 months later over half these children had made a full recovery, but a quarter was left with a major residual neurological deficit. Cerebral malaria in childhood may be an important cause of neurological handicap in the tropics.

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METEOROLOGICAL, VECTORIAL AND SOCIAL-ECONOMIC FACTORS RELATED TO MALARIA TRANSMISSION IN TIBET

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Malaria has been endemic in Linzhi Prefecture in the Tibet Autonomous Region (TAR) over the past 20 years, especially in Motou County with a highest incidence in the country in recent years. Considering spatial aggregation of malaria cases and specific vectors, the meteorological, vectorial and social-economic factors were analysed to determine the key factors related to malaria transmission in this particular area. Meteorological factors were incorporated in the spatio-temporal models. Seven models were established by Bayesian hierarchical models and Markov Chain Monte Carlo methods in comparison based on Deviance Information Criterion (DIC). In Tibet, malaria patients are scattered along the Brahmaputra River with spatial cluster, where inhabited by members of the Zang, Menba and Luoba nationalities. Relative humidity was the greatest influence factors, which affected the mosquito survival directly. The relationship between malaria incidence and rainfall was complex and it was not directly and linearly. The lags of temperature and relative humidity were similar and smaller than that of rainfall. Entomological investigation, which included adult anopheles collections, morphological and molecular identification was to identify the species of *Anopheles* including *An. maculatus* group, *An. peditaeniatus*, *An. barbumbrosus*, and *An. kochidonitz*. *An. pseudowillmori* was considered the sole malaria vector and the larval habitats only were paddy field. *An. pseudowillmori* accounted for 98.1% of the *Anopheles* composition. Human blood index, sporozoites natural infection rate, vector capacity and entomology inoculate rate were 29%, 0.56‰, 2.795 and 0.004389, respectively. Social-economic or household factors have been identified as increasing human-vector contact within a given ecological environment, which were collected by household investigation including the average income,domestic animals, the usage of bed-nets and malaria awareness. In Tibet, the risk of infection with malaria was high among residents of "poor" family without usage of bed-nets and few domestic animals. In conclusion, considering the unique and special characteristic of meteorological factors in Tibet, it may be speculated that the meteorological factors play major role in malaria transmission. Of course, vector species and abundance and social-economic status, are also known to have significant influence on the transmission of malaria.

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IDENTIFICATION OF MALARIA TRANSMISSION AND EPIDEMIC HOTSPOTS IN THE WESTERN KENYA HIGHLANDS: ITS APPLICATION TO MALARIA EPIDEMIC PREDICTION

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Malaria in the Western Kenya highlands is characterized by unstable and high transmission variability that causes epidemics during hyper transmission seasons. This study examined how terrain in the highlands affects the exposure and sensitivity of a site to malaria. The study was conducted in western Kenya highlands; two U-shaped valleys (Iguhu, Emutete), two V-shaped valleys (Marani, Fort-Ternan) and one plateau (Shikondi) for 16 months among 6-15 years old children. Malaria Exposure was tested using circum-sporozoite protein and merozoite surface protein immunochromatographic antibody test; malaria infection was tested by microscopic examination of blood smears, children's homes were georeferenced using global positioning system. Paired t-test was used to compare the mean prevalence rates of the sites. The mean antibody prevalence was 22.6% in Iguhu, 24% in Emutete, 11.5% in Shikondi, 8.3% in Fort-Ternan and 9.3% in Marani. The mean malaria infection prevalence was 23.3% in Iguhu, 21.9% in Emutete, 4.7% in Shikondi, 2.9% in Fort-Ternan and 2.4% in Marani. The difference in antibodies and malaria infection prevalence among the two valley systems and the plateau was significant ($P<0.05$). The difference in antibodies and malaria infection prevalence within the U-shaped valleys and within the V-shaped valleys was not significant ($P> 0.05$). There was clustering of malaria antibodies and infections around flat areas in the U-shaped valleys and random distribution of the infections in the V-shaped valleys and less clustered at the plateau. This study showed that the V-shaped and plateau ecosystems have low malaria parasites prevalence and few individuals with immune response to malaria parasites, they can be considered as epidemic hotspots. The U-shaped ecosystems are transmission hotspots.

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ANALYZING THE IMPACTS OF MALARIA INTERVENTION TECHNIQUES WITH A GIS-EQUIPPED, SPATIAL AGENT-BASED MODEL OF MALARIA

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My research is about spatial agent-based model (ABM) of malaria. We developed several ABMs of the vector dynamics lifecycle of malaria, and performed verification and validation of these. The ABMs were non-spatial: none of the agents possessed any spatial attributes. However, spatial heterogeneity is one of the most important factors for an effective representation of the mosquito environment. The dynamics of malaria can be affected by substantial local variations (e.g. locations of aquatic habitats and bloodmeal events) resulting from various spatial differences. Also, some events in the mosquito lifecycle (e.g. host-seeking, oviposition) are by nature spatial. A spatial ABM thus may provide opportunities for more realistic modeling of these events, and to obtain new insights from analyzing the spatial heterogeneity. We recently presented a spatial extension of the malaria ABM, and designed a landscape simulator to simulate landscapes used by the mosquito vectors. The landscapes, to be

used as inputs to the spatial malaria ABM, can be simulated with varying spatial heterogeneity of resources that are required by female mosquito agents to complete their gonotrophic cycles. The next step would be to augment the spatial ABM with georeferenced data. Several successful research efforts have shown the increasing use of GIS (Geographic Information System) and RS (Remote Sensing) for the study of spatial and temporal patterns of vector-borne diseases (including malaria). This would allow us to apply the ABM for a specific geographic location (e.g. some cluster of villages in Kenya). In association with the Center for Research Computing (CRC) at Notre Dame, we have collected data on various types of vector breeding sites, human habitats, and weather (primarily rainfall and temperature). Once the spatial ABM is run with these data, we can investigate the impacts of various intervention strategies for malaria. Out of many possible interventions, our primary emphasis is on habitat reduction, insecticide-treated bed nets (ITNs), and indoor residual spraying (IRS). Other than analyzing impacts of interventions, the ABM, equipped with georeferenced data, may also serve as a decision-making tool. For a particular region of interest, it can identify human populations at risk and the geographical spread of malaria. It can also help in stratifying malaria risk factors, and planning resource allocation more effectively.

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ASSESSMENT OF TREATMENT PRACTICES FOR MALARIA IN CHILDREN UNDER FIVE YEARS, NIGERIA, 2008

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Nigerian Demographic and Health Survey (NDHS) is a 5-year survey carried out to assess the impact of public health measures. Malaria is the commonest cause of febrile illness in Nigeria and *Plasmodium falciparum* accounts for >95% of these episodes. The 2002 drug efficacy study showed monotherapies, e.g. Chloroquine (CQ) and Sulfazodine-Pyrimethamine (SP) were no longer adequate for first line treatment. The reviewed national antimalarial treatment policy(2005) recommends the use of Artemisinin based-Combination Therapy (ACT) as the first line treatment for the management of malaria. The data of the 2008 NDHS was reviewed to assess the treatment practices for fever in children. We analysed NDHS data of 24,975 mothers whose children less than 5 years (U5) had fever 2 weeks preceding the survey and may have had antimalarial treatment.Descriptive analysis and comparison of treatment practices including promptness, type of drugs administered was conducted by chi-square test. Prompt treatment was defined as administration of antimalarial drugs within the first 24hours of the onset of fever.Altogether 3,968 (15.9%) children of 24,975 mothers interviewed had fever. Of the 3,968 children, 1,317(33.2%) received any type of antimalarial. Of the 3,968 children, 95 (2.4%) received ACT, 762 (19.2%) CQ and 234 (5.9%) received SP. Of the specific antimalarials administered, 41 (43.2%) children had ACT (n=95), 229(30%) had CQ (n= 762) and 80 (34.2%) had SP (n=234) readily available to their mothers at home ($p<0.01$). Of the 3,968 children, overall 595(15%) had prompt treatment with any antimalarials, 71(1.8%) received prompt treatment with ACT, 349(8.8%) had CQ and 99(2.5%) had SP. Children of mothers with at least secondary education were more likely to receive prompt treatment ($p<0.01$) and more likely to receive CQ and SP rather than ACT ($p<0.01$). Prompt treatment of fever and the use of the recommended antimalarial (ACT) by mothers of U5 is low. There is a need to sensitise mothers on prompt and appropriate treatment of febrile episodes in children with ACT.

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IMPACT OF HEALTH RESEARCH CAPACITY STRENGTHENING IN LOW AND MIDDLE INCOME COUNTRIES: THE CASE OF WHO/TDR PROGRAMS

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Measuring the impact of capacity strengthening support is a priority for the international development community. Several frameworks exist for monitoring and evaluating funding results and modalities. We report on the impact of individual and institutional capacity strengthening programmes conducted by the UNICEF/UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR) and on factors that influenced the outcome of its Research Capacity Strengthening (RCS) activities. Quantitative and qualitative methods (questionnaires and in-depth interviews) were applied to a group of 128 individual and 20 institutional capacity development recipients that completed their projects between 2000 and 2008. A semi-structured interview was conducted on site with scientists from four institutions. Most grantees, both individual and institutional, reported beneficial results from their grants. However, glaring inequities stemming from gender imbalances and a language bias towards English were identified. The study showed that skills improvement through training contributed to better research proposal formulation, but not necessarily to improved project implementation or results communication. Appreciation of the institutional grants' impact varied among recipient countries. The least developed countries saw the grants as essential for supporting basic infrastructure and activities. Advanced developing countries perceived research grants as complementary to available resources, and particularly suitable for junior researchers who were not yet able to compete for major international grants. There is need for a more equitable process to improve the effectiveness of health research capacity strengthening activities. Support should be tailored to the existing research capacity in disease endemic countries and should focus on strengthening national health research systems, particularly in the least developing countries. Stakeholders' engagement at country level would facilitate design of more specific and comprehensive strategies based on local needs.

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THE GENETIC RELATEDNESS OF *PLASMODIUM FALCIPARUM* PARASITES *VIS A VIS* THEIR SPATIAL DISTRIBUTION

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Understanding the genetic relatedness of *Plasmodium falciparum* parasites is important in providing insight on how these parasites are being transmitted in various localities. In this study: 1. Since *P. falciparum* mixed clone infections are common in nature, PyrosequencingTM was validated as a technique enabling the identification of each genetically distinct clone represented in an infection by assigning proportions to the SNPs representing each genetically distinct clone and enabling the identification of parasite genotypes in every isolate analysed; and, 2. The genetic relatedness between the identified clonal genotypes was determined. These results comprise a total of 58 samples; 8 samples collected from Cameroon, 15 from Kenya and 35 from Mali. The data consists of 6 SNPs analysed by PyrosequencingTM. 83 clonal genotypes were identified by PyrosequencingTM from the analysed isolates. Genetic relatedness (GRs) was determined and Pairwise comparisons conducted between clones occurring (i) within an isolate i.e. a major and minor clone (ii) between isolates within the countries and, (iii) between isolates from the different countries. The results indicated that parasites within one isolate in a polyclonal infection were found to have higher GR compared to parasites from another isolate within a region and beyond it. This offers the possibility that parasites occurring within households and in close neighbourhoods would be more closely related than those separated by large geographic distance. On this basis, it is recommended that a larger

study should be conducted to determine the level of genetic relatedness in parasites collected within households and in close neighbourhoods to clearly establish the level of genetic relatedness of these parasites in natural populations. This information would enable the detection of foci of malaria transmission which is important for effective deployment of malaria interventions.

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FEVER AND PARASITE DENSITY IN PASSIVE CASE DETECTION: IMPLICATIONS FOR ENDPOINTS USED IN MALARIA VACCINE TRIALS

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The measurement of clinical malaria is complicated in high transmission areas where parasitemia during a febrile presentation may be incidental. Fever at the time of presentation and parasite density cut-offs derived from attributable fractions of fever due to malaria among the general community have been used to refine case definitions. However, whether these criteria are valid in passively detected cases, who have histories of fever, is not known. We measured the proportion of ill patients presenting with current fever (≥37.5°C) and estimated the effect of parasite density on fever during home and clinic-based care from 2006-2009 in a vaccine trial site population of 2,204 people residing in eight forest villages of Orissa, India. We modeled the odds of fever as a function of continuous parasite density by age category and adjusted for year, village, month, sex, and correlated observations using unconditional logistic regression and general estimating equations. 4,889 screenings for malaria among 1,493 ill persons were conducted. The prevalence of current fever was 45% and 52% among those with parasitemia. Parasite prevalence and the incidence of malaria, 39% and 215/1000years respectively, varied with age. Mean parasite density decreased with age and increasing densities were associated with higher odds of current fever (p for trend<0.0001). The unadjusted odds ratio (OR) of fever for a one log increase in parasite density was 1.06 (95%CI: 1.04, 1.07); including interaction between parasite density and age and adjusting for village, month and intra-subject correlation the OR of fever was 1.14 (95%CI: 1.08, 1.21). Overall, 28% (95%CI: 26, 29) of current fevers were attributable to malaria. A parasite density cut-off of 500 provided 87% sensitivity and 84% specificity in classifying malaria-attributed current fever. Increasing parasite density increased the odds of current fever in forest areas of India. In the context of persons who sought screening because they were ill, the fraction of current fevers attributable to malaria was low in spite of high malaria transmission. The results of this study, which was akin to a vaccine trial using passive case detection such as Phase 3 RTS,S, suggest that in order to preserve power and reduce bias the primary endpoint should not use fever at the time of presentation or parasite density cut-offs as case definition criteria.

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SCALING UP THE IMPLEMENTATION OF HMM WITH THE USE OF RDT IN SARAYA HEALTH DISTRICT: FEASIBILITY, PHARMACOVIGILANCE, HEALTH SEEKING BEHAVIOR AND IMPLICATIONS FOR SURVEILLANCE SYSTEM

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From 2005 and 2008 malaria morbidity and mortality have respectively dropped from 32, 5% and 20,6% to 5,6% and 7,1% in Senegal health services ; data did not include the underserved communities in a context of 70% of the population living at least at 15 kilometers from the nearest health unit. To fill this gap Home Management of Malaria has been scaled up in this district through Community Health Workers and malaria volunteers. The objective of the study is to assess HMM and enhance its surveillance system. Following a community census and a survey on CHW and volunteers' profiles, a baseline household cluster survey including KAP and Health Seeking Behavior has been completed. CHW and volunteers have been trained on the use of RDT, Artemisinin-based Combined Therapy administration and malaria pharmacovigilance. Supervisions were held to follow the process in 47 community health units. 57% of community agents are CHW, 43% are malaria volunteers. The mean age is 33 years, 14% are illiterate, 74,5% have previously involved in malaria treatment, 40% have traditional healers in their area. The KAP survey has concerned 981 households; the respondents' mean age is 56 years, 86% are male, 69% are farmers, and 22% are literate. Among them 84% are aware of malaria transmission, 72% recognize malaria symptoms, 94% know RDT as a confirmation tool and 68% LLINs as protective. Treatment by ACT and quinine is known by 41%, 39% are aware on adverse events. 91 households (9.3%) have responded to the health seeking behavior survey 14% are close to traditional healers among these latter 16% are "treating" malaria. From June 2010, in a 10050 population, 3699 consultations have been completed at the community level; 59% of clinical malaria confirmed by RDT, 23.6% negative and 0.03 invalid; 865 referrals completed, 12 adverse events and 29 deaths notified. Large scale implementation of HMM is feasible and useful for malaria surveillance; it is fundamental to follow up RDT and data quality, outcomes of executed referrals, and the potential role of traditional healers.

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ESTIMATING THE CLINICAL BURDEN OF *PLASMODIUM FALCIPARUM* MALARIA IN INDONESIA IN 2010

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The contemporary map of population at risk of *Plasmodium falciparum* in Indonesia estimated that 132.8 million (57.1%) of the population lived at risk of *P. falciparum* transmission in 2010. Of these, 70.3% inhabited areas of unstable transmission and 29.7% in stable transmission zones. However, the burden of *P. falciparum* in this archipelago has not been estimated. The new cartographic technique for deriving the clinical burden estimates of *P. falciparum* developed by Malaria Atlas Project (MAP, http://www.map.ox.ac.uk) was used in this study. The mapped of unstable and

stable *P. falciparum* malaria transmission was first determined. Estimates of the plausible incidence range of clinical cases were then calculated within the spatial limits of unstable transmission. A modelled relationship between clinical incidence and prevalence was used to estimate incidence areas of stable transmission. Geostatistical joint simulation was used to quantify uncertainty in these estimates at provincial scales. These results are summarized across main islands of Indonesia and the implications for evaluation of malaria elimination elaborated.

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AGE AND SEX DISTRIBUTION OF GAMETOCYTE-POSITIVE INDIVIDUALS IN SOUTHERN ZAMBIA

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The incidence of malaria has decreased in Zambia as a result of malaria control interventions, including increased coverage with insecticide treated nets (ITNs), indoor residual spraying (IRS) and artemisinin-combination therapy (ACT). Gametocytes are the sexual stage of the malaria parasite critical to transmission. Malaria is commonly diagnosed based on the presence of asexual parasites and most anti-malarial drugs act on these stages. However, of importance to malaria control and elimination is the prevention of gametocytemia. The present study compares the age and sex distribution of gametocyte-positive individuals in 2007, 2008 and 2009 malaria transmission seasons in rural southern Zambia. A cross-sectional survey of individuals residing in randomly selected households was conducted in Choma District, Southern Province, Zambia. A total of 174, 317 and 675 blood samples were collected in 2007, 2008 and 2009, respectively. The samples from 2007 were examined by microscopy after making thick and thin films. Samples collected in 2008 and 2009 were assayed using RT-PCR to detect the pfs25 mRNA expressed in *P. falciparum* gametocytes. The proportion of individuals with detectable gametocytes was 2.8% in 2007 by microscopy, and 4.5% and 1.5% by RT-PCR in 2008 and 2009, respectively. There were no significant differences in gametocyte positivity between males and females in any year. Gametocytemia was more frequent in the 5-20 year age-group among the 2008 and 2009 cohorts, with 79% and 90% of gametocyte-positive individuals within this age group, respectively. In contrast, 60% of gametocyte-positive individuals were children younger than five years of age in 2007. The prevalence of gametocytemia decreased over the three years, with the lowest prevalence of gametocytemia recorded in 2009, concurrent with the scale-up of malaria control interventions. The prevalence of gametocytemia was highest in school-age children and young adults as malaria transmission decreased, facilitating targeted treatment in schools.

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ANTIBODIES TO *PLASMODIUM FALCIPARUM* IN A REGION OF DECLINING MALARIA TRANSMISSION IN SOUTHERN ZAMBIA

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The prevalence of malaria has decreased dramatically in southern Zambia due to high coverage with insecticide-treated nets and artemesinin-combination therapy. We assessed the prevalence of antibodies to *Plasmodium falciparum* in this region of declining malaria transmission to investigate whether the prevalence of seropositivity would decrease concurrently. Participants were residents of randomly-selected households in the catchment area of Macha Hospital in Choma District, Southern Province, Zambia. Residents of some households participated once (cross-sectional households) and others were assessed at repeated visits (longitudinal households). Samples were tested for the presence of *P. falciparum* antigen using a rapid diagnostic test. An enzyme immunoassay was used to measure IgG antibodies to whole *P. falciparum* asexual stage parasites. Seropositivity was defined based on a threshold value established using plasma from persons never exposed to malaria. A total of 433, 742 and 822 blood samples were collected from participants in 2008, 2009 and 2010, respectively, for the cross-sectional survey. A total of 118, 150 and 185 participants were recruited into the longitudinal cohort in 2008, 2009 and 2010, respectively, of whom 39, 36 and 47 had complete follow-up. The parasite prevalence decreased from 8.1% in 2008 to 0.24% in 2010 within the cross-sectional cohort and from 3.4% in 2008 to 0.54% in 2010 within the longitudinal cohort. The prevalence of seropositivity, however, did not decrease and was 47.8%, 69.3% and 68.4% in 2008, 2009 and 2010, respectively. Mean OD values in the cross-sectional cohorts similarly did not decrease (0.634, 0.958 and 0.904 respectively). In general, seropositivity increased with age and was higher in females than males. Although the parasite prevalence declined over the study period from 2008 to 2010, seroprevalence to whole *P. falciparum* antigens remained high. Serology is a useful marker of exposure to *P. falciparum*, but antibodies to whole parasite antigens are not a sensitive indicator of recent decline in parasite prevalence.

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KNOWLEDGE, ATTITUDE, AND PRACTICES (KAP) REGARDING MALARIA IN MUMBAI, INDIA

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According to the National Vector Borne Disease Control Program, malaria incidence has been increasing in India and we hypothesize that the most important reason for this rise is the lack of appropriate human behavior in preventing malaria. This study was thus focused on studying the relationship between various behavioral risk factors and malaria infection in and around Mumbai, India. Four cluster groups in and around Mumbai were identified and a total of 30 households were selected from each cluster. Cluster groups were divided into urban middle class (subjects living in city apartment complexes), urban lower class (subjects living in slums), immigrants (construction workers living at construction sites) and rural lower class (subjects living in a village near Mumbai). Relative poverty between regions is city<slums<Construction workers<village. A structured questionnaire focusing on socio-demographic factors, environmental factors, malaria knowledge, malaria preventive practices and any previous malaria infection was administered to the eligible participants by a trained interviewer. Malaria varies by regions (City: 77% of respondents (or a member of their family) have had malaria, Construction workers: 67%, Village: 17%, Slums: 60%, $p<0.001$). Reported knowledge also varies by region (City: average of 80% of knowledge questions correct, Slums: 73%, Construction workers: 67%, Village: 62%, $p<0.001$). Malaria status appears to be related to knowledge, with those reporting having had malaria scoring higher on the knowledge test (average 55% correct) compared to those reporting never having had malaria (average 45% correct, $p=0.029$). Mosquito nets usage in Construction workers showed 83 % usage while city did not use it (Construction workers: 83%, Village: 24%, Slums: 13%, City: 0%). The usage of insecticide treated nets were mostly seen in Slums (13%) and Village (3%) while other regions did not report any usage. Low rates of reported malaria in the village may reflect a lower chance of surviving malaria. Lack of malaria knowledge may adversely affect this group's ability to recognize and report malaria symptoms and take preventive measures. Thus, our study highlights an urgent need to incorporate malaria education as well as use of insecticide treated nets as an integral measure for all malaria control programs from national to local levels.

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According to the National Vector Borne Disease Control Program, malaria incidence has been increasing in India and we hypothesize that the most important reason for this rise is the lack of appropriate human behavior in preventing malaria. This study was thus focused on studying the relationship between various behavioral risk factors and malaria infection in and around Mumbai, India. Four cluster groups in and around Mumbai were identified and a total of 30 households were selected from each cluster. Cluster groups were divided into urban middle class (subjects living in city apartment complexes), urban lower class (subjects living in slums), immigrants (construction workers living at construction sites) and rural lower class (subjects living in a village near Mumbai). Relative poverty between regions is city<slums<Construction workers<village. A structured questionnaire focusing on socio-demographic factors, environmental factors, malaria knowledge, malaria preventive practices and any previous malaria infection was administered to the eligible participants by a trained interviewer. Malaria varies by regions (City: 77% of respondents (or a member of their family) have had malaria, Construction workers: 67%, Village: 17%, Slums: 60%, p<0.001). Reported knowledge also varies by region (City: average of 80% of knowledge questions correct, Slums: 73%, Construction workers: 67%, Village: 62%, p<0.001). Malaria status appears to be related to knowledge, with those reporting having had malaria scoring higher on the knowledge test (average 55% correct) compared to those reporting never having had malaria (average 45% correct, p=0.029). Mosquito nets usage in Construction workers showed 83 % usage while city did not use it (Construction workers: 83%, Village: 24%, Slums: 13%, City: 0%). The usage of insecticide treated nets were mostly seen in Slums (13%) and Village (3%) while other regions did not report any usage. Low rates of reported malaria in the village may reflect a lower chance of surviving malaria. Lack of malaria knowledge may adversely affect this group's ability to recognize and report malaria symptoms and take preventive measures. Thus, our study highlights an urgent need to incorporate malaria education as well as use of insecticide treated nets as an integral measure for all malaria control programs from national to local levels.

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REINTRODUCTION OF *FALCIPARUM* MALARIA IN THE NORTH COAST OF PERU, 2010 - 2011

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In Peru, the number of cases of *falciparum* malaria has decreased in the last years and the last case in the North Coast was reported in 2006. After the identification of two cases of *falciparum* malaria in Tumbes in October 2010, an investigation was conducted to confront this reintroduction in the North Coast of Peru.The study was led by the Peruvian Ministry of Health. After identifying new cases, cross-sectional case-finding studies were conducted in the surrounding areas. Cases were diagnosed by smear microscopy and confirmed by nested-PCR when whole blood was available. Genotyping was conducted using microsatellite molecular markers. An epidemiological questionnaire was applied to all cases. Five 12-hour live bait (human landing) mosquito collections took place in two locations where initial cases presented. Twenty-three cases were identified between October 2010 and April 2011, and nine cases were confirmed by nested-PCR (39%). Almost 1200 people were evaluated in areas surrounding the case houses searching for other febrile cases; two additional cases were found. All cases were symptomatic and the most common symptoms were fever and headache (95%), followed by muscle pain (90%), chills (90%), and sweating (80%). No cases had a severe presentation. On average cases were 28 yo, 55% were male and only two reported a previous malaria episode (11%). More than half did not use bed nets (53%) and, 74% lived with people with malaria-like symptoms. The mean time before seeking treatment was 11 days (0-48 days). Twenty-two cases were treated with artesunate/mefloquine (96%); one pregnant woman was treated with quinine. All the subjects reached clinical cure and none reported side effects. No cases have been reported in neighboring regions of Piura, Peru and Ecuador to date. We could not differentiate strains between all six isolates genotyped and we found high similarity to a strain circulating previously in Peruvian Amazon Basin. We collected 194 mosquitoes but only 34 adults of *Anopheles albimanus* were found in the locations assessed. In conclusion, this outbreak demonstrates the latent, permanent risk of reintroduction of *falciparum* malaria in the North Coast of Peru and suggests that transmission continues to date. Better clinical, epidemiological and control data will facilitate control measures executed by Peruvian authorities.

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IDENTIFICATION OF RISK FACTORS FOR MALARIA INFECTION IN MACHA, ZAMBIA DURING THE DRY SEASON FOR EPIDEMIOLOGICAL MODELING AND OUTBREAK PREDICTION

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Current control methods for malaria in Southern Africa include indoor residual spraying and insecticide-treated bed nets which have been responsible for the reduction of malaria transmission in some endemic areas by up to 80 percent. However, these tactics do little to remove asymptomatic cases from the environment. Particularly in areas of unstable transmission, asymptomatic cases act as reservoirs that sustain malaria through the dry season, facilitating transmission the next year. Asymptomatic infections tend to cluster; by detecting and determining risk factors for these foci, local outbreaks of malaria can be predicted, enhancing elimination of asymptomatic cases. Detection can be accomplished by implementing both active and passive surveillance plans, including the use of Rapid Diagnostic Tests and routine collection of malaria case data by a central location. Current work in Macha, Zambia has demonstrated that malaria incidence data can be collected in real time through the use of RDT and Short Message Service data sent via cell phone. Symptom-free household members of malaria-positive cases are tested for infection to detect asymptomatic carriers, and a Global Positioning System coordinate is taken at the homestead. Currently lacking is a means to standardize the data collection and mobilize epidemiological methods, such as Geographical Information Systems (GIS), to develop surveillance frameworks that can be incorporated into a national strategy for malaria detection. We report on a procedure to determine appropriate data stratifications to form site-specific malaria surveillance systems for risk-factor analysis in the Macha area of Zambia serviced by 12 rural health clinics. Homesteads of positive malaria cases were visited and the ecologic, demographic, and socioeconomic status of each was recorded. These data are used in conjunction with GIS and malaria outbreak analysis to determine risk factors for malaria and to develop the framework necessary to implement an effective and efficient malaria surveillance system.

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DECLINING BURDEN OF MALARIA IN A RURAL COMMUNITY IN MUHEZA DISTRICT NORTHEASTERN TANZANIA OVER A PERIOD OF 18 YEARS (1992-2010) AND ITS IMPACT ON ANTIMALARIAL PRESCRIPTION

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The declining burden of malaria in some endemic countries indicate that most of febrile cases may not necessarily be due to malaria; thus, over-diagnosis and -prescription of anti-malarials may pose an increasing challenge to the health system. This study was conducted to assess the changing burden of malaria and examine the effect of decreasing malaria prevalence on dispensing of anti-malarials. Blood smears were prepared from finger prick/venous blood and examined for malaria parasites by microscopy during cross-section surveys conducted between September 1992 and June 2010 in two villages (Magoda and Mpapayu) in Muheza district, Tanzania. Prevalence of *Plasmodium falciparum* infections was compared across the years and study villages. Data from patients treated for malaria were obtained from health records at Magoda dispensary (without diagnostic laboratory facilities) and Mkuzi Health Centre (with capacity for malaria diagnosis by microscopy which serves as a referral centre for the study villages) and used to assess the level of antimalarials dispensing at the two health facilities. The prevalence of *P. falciparum* infections in Magoda village decreased significantly from 83.5% in 1992 to 15.0% in 2010 and in Mpapayu, the prevalence dropped from 83.3% in 1998 to 11.7% in 2010. Spleen rate (from >40% to <1%), anaemia prevalence (69% to <25%) and gametocyte rates (23% to <1%) also declined over the same period. From January 2008 to December 2010, a total of 5755 patients were attended and treated presumptively for malaria with Artemether/Lumefantrine at Magoda dispensary, and the number of patients increased from 114 in January 2008 to 289 patients in October 2010. At Mkuzi, over 50% of patients (n=564) with negative test results by microscopy were prescribed with anti-malarial drugs. Although a remarkable decline in the burden of malaria occurred between 1992 and 2010, the number of patients treated with antimalarial drugs in the same area did not decline, leading to over-prescription of anti-malarials. Accurate diagnosis and treatment of patients with positive malaria results is urgently needed to target anti-malarials to patients with malaria, reduce wastage of expensive drugs and improve management of other causes of febrile infections.

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PYRETHROID RESISTANT *CULEX QUINQUEFASCIATUS:* AN OBSTACLE TO THE USE OF INSECTICIDE TREATED NET?

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In many West African countries, human activities often create mosquito breeding sites, such as improper drainage systems or choked gutters that hold water that *Culex quinquefasciatus* especially like to breed in. This problem has not been adequately addressed because *Cx. quinquefasciatus* are currently not of much importance as disease vectors in most of these countries. However, some studies have suggested their possible negative effect on the use of insecticide-treated nets, especially when resistant to pyrethroid insecticides. As a result, efficacy of alphacypermethrin treated net were evaluated in tunnel test bioassay. A 6-week trial was further conducted in experimental huts to assess its entomological impact on wild *Cx. quinquefasciatus* in Southern Benin. In tunnel test, 40mg/m^2 of alphacypermethrin treated netting induced <15% mortality, however it was effective in preventing blood feeding (50-70%). Similar trend were observed in the experimental hut, low mortality rate (23.8%) but a marked inhibition in blood feeding rate (81.2%). Relative to the number of mosquitoes exiting naturally into the verandah of control hut, alphacypermethrin treated net induced exophily of the pyrethroid-resistant *Cx. quinquefasciatus* into the verandas of the huts. Pyrethroid insecticide treated nets may still offer protection to users against pyrethroid resistant *Cx. quinquefasciatus*. Nevertheless, combination of insecticide treated nets and other mosquito strategies may be require to manage their populations.

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FIELD EVALUATION OF AN ATTRACTANT (OVIPOSITION PHEROMONE IN COMBINATION WITH INSECT GROWTH REGULATOR) FOR SURVEILLANCE AND CONTROL OF DENGUE AND CHIKUNGUNYA IN KERALA, INDIA

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Dengue has been known to be endemic in India as a benign and self-limited disease, the principal vector of which is *Aedes aegypti*. In recent years, the disease has changed its course manifesting in the severe form as DHF, with increasing frequency of outbreaks in many urban and rural parts of India. Similarly, chikungunya is another illness caused by chikungunya virus (CHIKV), transmitted by *Ae. aegypti*. In India, chikungunya re-emerged after a lapse of three decades in a virulent epidemic form in late 2005. In 2006, a total of 1.39 million suspected cases from 213 districts in 15 states and about 565.42 million people were at the risk of infection. Kerala state reported 70,731 suspected cases mainly from three coastal districts viz., Alappuzha with a maximum of 58,308 (82.44%), Thiruvananthapuram with 8,311 (11.75%) and Eranakulam with 1,840 (2.60%) mostly confined to urban areas including small townships. A longitudinal evaluation was undertaken during 2009 - 2010 to assess the field efficacy of attracticide in Kadakkarapally and Vettackal of Alappuzha district, Kerala, India. 216 houses (951 population) were selected and 748 ovitraps (control and experimental) were placed both inside and outside the house. Monitoring of ovitraps was done on weekly basis and the ovitrap positivity in experimental and control bowls in Vettackal ranged from 14.5 to 55% and 9.9 to 46.7% respectively. The corresponding results in Kadakkaraplly were 19.8 to 40.8% and 15.4 to 36.5%. Oviposition active index (OAI) of the pooled data ranged from 0.2 to 0.5 indicating effective attractant property of the compound. The study clearly indicated that the compound lured Aedes mosquitoes to oviposit in ovitraps placed inside and outside the house. Besides, the increase in number of eggs in experimental bowls revealed pheromonal action of the compound and its feasibility as an effective tool in surveillance and control of Aedes mosquitoes in Kerala, India.

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MODELLING OF THE EFFICIENCY OF THE INSECTICIDE FENITROTHION ON THE *ANOPHELES GAMBIAE* TO MALANVILLE

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The control methods used against mosquitoes nowadays are the effective ways of protection against malaria in Africa. The control of the vector is however subject to increasing problems of resistance of the wild anopheles to conventional insecticides. This has led the IRD and WHO to test the quality of new formulations of the insecticide in indoor residual spraying in Malanville (North Benin). Fenitrothion has presented five forms according to the dose, the liquid or the powdered nature; these have been evaluated in six experimental huts belonging to the Anopheles Biology and

Control (ABC) network. A longitudinal study was carried out to evaluate the product according to four entomological criteria, among these; two have the characteristics that are essentially deterrent and lethal. So we have focused our research work on these two aspects. As far as this study is concerned, 2735 anopheles were caught in six trap-huts for six months. Our objective was to determine in what form (liquid or powdered) the insecticide was more effective at limiting the number of mosquitoes entering the huts and kill them. To do this, we used two tests including the comparison, the nonparametric Kruskall-Wallis' test (to determine if there is a significant difference in terms of the median among the six trap-huts of the study) which one is added to two by two comparison's test and the equality of several proportions with Holm correction (to establish a significant difference in terms of the death proportion in the six trap-huts). Then two generalized linear models including mixed effect (with random variable, day) for deterrent criteria were established to compare which of the five trap-huts has repelled or has effectively killed mosquitoes. An observation of significant differences between the numbers of mosquitoes captured and their survival in the control hut in relation to the treated huts, we can thus conclude that the mixed Poisson model explains better the phenomenon of deterrence while the joint logistics best explains the phenomenon of survival of mosquitoes in the treated cells.

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EVALUATION OF HUMAN EXPOSURE TO *AEDES ALBOPICTUS*, TOWARD A BIOMARKER OF VECTOR CONTROL EFFICACY

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The global expansion of *Aedes albopictus* stresses the need to improve current entomological methods. Recent findings suggest that human antibody (Ab) response to arthropod salivary proteins can provide new tool for monitoring vector populations. In La Reunion urban area, *Ae. albopictus* is the only anthropophilic *Aedes* specie. We aim to evaluate human IgG response to *Ae. albopictus* salivary proteins in order to get an insight of the adult population exposed to *Ae. albopictus* bites as well as the efficacy of vector control program. We measured the IgG response to *Ae. albopictus* saliva before (T0), two (T+2), four (T+4) and six (T+6) weeks after deltamethrine pulverisation. The use of individual protection devices was investigated and the abundance of *Ae. albopictus* adult female population was monitored. At T0, the immunoassays indicate high prevalence (83%) of IgG anti *Ae. albopictus* saliva which is consistent with the high density of adult female population. This Ab response is maintained at T+2 and T+4 while significant decrease is observed at T+6 (61%) coinciding with the decline of *Ae. albopictus* population. Additionally, the level of IgG anti saliva is lower in individuals using an individual protection compared to those unprotected (P=0.034). We assessed the Ab cross reactivity between *Ae. albopictus* and *Ae. aegypti* saliva. Low level of IgG cross reactivity is noticed between these two closely related species, underlining the specificity of the Ab response. These results suggest that IgG to *Ae. albopictus* saliva could be a potential biomarker of exposure to *Ae. albopictus* bites and a direct tool for the evaluation of control strategies; therefore, allowing to assess accurately the risk of *Ae. albopictus*-borne diseases transmission. To improve the reproducibility, the identification of specific *Ae. albopictus* salivary protein is under investigation.

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DEVELOPMENT OF ALLELE-SPECIFIC LOOP-MEDIATED ISOTHERMAL AMPLIFICATION METHOD (AS-LAMP) FOR DETECTION OF THE L1104F KDR MUTATION IN *ANOPHELES GAMBIAE* S.L

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The knock down mutation (kdr) in Western Africa is due to a substitution of the Leucine (L) by the Phenylalanine (F) in the position 1014 of the sodium channel gene sequence. This mutation induces resistance to pyrethroids and impacts negatively vector control against *Anopheles gambiae* s.l. The management of resistance to insecticides requires simple and effective tools for its early detection and for early decision-making. Our study aimed to develop simple and cost/effective method to detect the West African-type *kdr* mutation in field collected mosquitoes. Specific primers to detect the mutation have been designed with the mutation on the 5' end of the BIP primer to allow distinguishing between the resistant type (L1014F) and the sensitive type (L1014L) of the kdr mutation. Genomic DNA of three mosquitoes homozygous (L1014F/L1014F), heterozygous (L1014F/L1014L) and homozygous (L1014L/L1014L) confirmed by DNA sequencing has been used as template to set

the reaction conditions. The reaction has been processed in a real time turbidimeter at 63°C for 75 min. The reaction has been detected by the turbidity values as well as by naked eye. The sensitivity, the specificity of this method has been compared to the DNA sequencing method using 120 field-collected mosquitoes. The detection time for the L1014F/L1014L and L1014L/L1014L genotypes were around 62 min and 75 min respectively using L1014L type primers. Using these primers, there is no amplification for the resistant type untill 75 mins after incubation. For the resistant genotype detection, the amplification starts around 60 min and 65 min after incubation and there is no amplification for the sensitive type until 75 min when using the resistant type primers. The specificity and the sensitivity of the AS-LAMP compared to the DNA sequencing were respectively 0.92 (CL: 0.74 - 0.98) and 0.99 (CL: 0.94 - 1) This AS-LAMP method can be performed using minimum equipment like a water bath at 63°C. The reaction result is detectable by naked eye due to the deposit of magnesium pyrophosphate a by-product of the reaction. AS-LAMP can be use for *kdr* mutation detection for earliest decision-making in the context of less equiped laboratory.

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FIELD USE OF GENETICALLY ENGINEERED (GE) MOSQUITOES

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Recent advances in insect genetic engineering have opened new possibilities for the control of mosquitoes and hence of mosquito-borne diseases. Oxitec has developed engineered strains of *Aedes aegypti* and *Ae. albopictus* which are homozygous for one or more dominant lethal genes and are "genetically sterile" unless provided with the repressor molecule tetracycline in the diet. Use of such strains for mosquito control, a method known as RIDL, is based on the Sterile Insect Technique (SIT) which has been used successfully since the 1950s for the area-wide suppression or elimination of several major agricultural pest insects. Sterile males are released continually over a wide area to mate with the target pest population; no progeny result from these matings and the target population declines. Engineered strains with the necessary genetic properties ('RIDL strains') have been constructed. Field trials of the lead strain of *Ae. aegypti*, OX513A, have been initiated in several countries. This followed extensive testing in contained conditions and mathematical modeling to predict the outcome both of such trials and of potential programmatic use. Such models indicate that this would be both effective and cost-effective as a dengue control strategy, potentially as a stand-alone method but even more so if integrated with other existing methods such as larviciding and source reduction. This presentation will summarise the results of experiments to date and discuss the options for further testing and programmatic use of such technology.

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INCREASE IN SPOROZOITE RATES AND KDR ALLELE FREQUENCIES AFTER INITIATION OF IRS AND ITN INTERVENTIONS IN CONTINENTAL EQUATORIAL GUINEA

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Anti-vector approaches reliant on insecticides may be compromised as a result of selection for target site mutations conferring resistance to pyrethoid and carbamate-class insecticides. In 2007, The Equatorial Guinea Malaria Control Initiative (EGMCI) was initiated in the continental region of Equatorial Guinea with funding from the Global Fund. The EGMCI comprises a comprehensive anti-malaria program with a strong emphasis on anti-vector interventions consisting of multiple rounds of indoor residual application of pyrethroids, a single round of a Bendiocarb as well as distribution of deltamethrin-treated bed nets. Entomological monitoring provides important data on vector abundance, sporozoite infection levels and insecticide resistance alleles. We analyzed 1,462 *Anopheles gambiae* mosquitoes from nine sentinel sites prior to the start of the intervention activities in 2007 and 3,261 mosquitoes from eleven sites in 2009-10 after the start of intervention activities. Conventional and quantitative PCR assays were employed to determine species and molecular form identification, sporozoite rates, *kdr* and *ace*-1 (carbamate target site mutation) allele detection in individual mosquitoes. Significant increases in *kdr* allele frequencies were detected in seven of the nine sites for which pre- and on-going intervention data were analyzed. Sporozoite incidence in sentinel sites increase between 2007 and 2010 and this change was highly significant. To date, no *ace*-1 alleles have been observed in any sentinel site. In conclusion, prior to the initiation of intervention activities, both *kdr* alleles were present in all sentinel sites with the L1014F allele occurring at significantly higher frequencies than the L1014S and wildtype alleles. The dramatic increase in *kdr* allele frequencies since the start of anti-vector interventions is likely due to the selection pressure imposed by the intensive use on pyrethroids as part of the IRS and LLIN distribution programs. Given the absence of *ace*-1 alleles, carbamate insecticides would be a suitable choice for continued IRS applications. Significant increases in sporozoite rates were observed in five of the six sites for which paired data was available. Although no comparative data on mosquito abundance before and after control is available, these results suggest that the risk of infection to humans remains high despite ongoing anti-vector activities.

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PUBLIC POLICY FAILURES AND THE DEVELOPMENT OF NEW INSECTICIDES

Richard Tren[1], Kimberly Hess[1], Donald Roberts[2]

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Public health insecticides (PHIs), on netting or sprayed on walls, are vital to control vector-borne diseases and are the primary means of preventing such diseases. The current arsenal of PHIs for malaria control is limited to just 12 old chemicals, most belonging to one class (pyrethroids). This is the direct result of environmentalist opposition and misinformation campaigns, stifling regulatory hurdles, weak public health advocacy, limited commercial markets, and activism by some United Nations agencies; conditions which are worsening. While the World Health Organization (WHO) Global Malaria Program and Roll Back Malaria Partnership recognize the need for new PHIs and are beginning to take steps to help encourage investment, other UN agencies are conducting a global campaign to eliminate the use of PHIs. This old campaign was reinvigorated in 1997 when the World Health Assembly passed resolution 50.13 calling on member countries to reduce their reliance on PHIs. As a continuation of this campaign, the UN Environment Program (UNEP), the Global Environment Facility, the Stockholm Convention Secretariat (SCS), and environmental sectors of WHO and the Pan American Health Organization falsely claimed success of 'environmentally sound malaria control interventions' in Mexico and Central America without PHIs, specifically DDT. Additionally, even though the Stockholm Convention provides an exemption for use of DDT until a safe, effective and affordable alternative is available, the SCS has proposed plans to eliminate use and production of DDT by 2020. In response and to ensure continued availability of DDT to malaria programs where it is needed, the Southern African Development Community has officially announced intent to begin local production of DDT. The fact that this region has committed to producing a PHI in the 21st century that was first created in the late 1800's, highlights severe regulatory and policy flaws governing insecticide

development and disease vector control. Also it prominently displays the environmental movement's war against control programs in disease endemic countries.

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NOVEL TECHNIQUES FOR EVALUATING SPATIAL REPELLENTS

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Spatial repellency which refers to the ability of an insecticide which in the vapour state, prevents mosquitoes from gaining access to the host and inhibit blood feeding, is gaining considerable attention in the recent past. There is a slow paradigm shift from dependence upon the toxicity of insecticides as the only mode of action towards mosquitoes that transmit malaria. Evidence in the past shows that the main method by which DDT reduced malaria prevalence in most parts of the world was by its spatial repellency characteristic. The guidelines put in place by the World Health Organization for evaluating spatial repellents are not sufficient and do not encompass most aspects of spatial repellents. In this study we have developed novel throughput semi-field systems and full field experimental hut assays for quantifying the main outcome indicators of spatial repellents which include; deterrence, irritancy/excito-repellency, feeding inhibition, effective dose and distance of spatial repellents and mortality. We have compared transfluthrin and metofluthrin coils to DDT which is the gold standard spatial repellent. The techniques used for testing in this study are safe, capture all outcome indicators for testing spatial repellents and are a reasonable representation of what goes on in the real world where spatial repellents are used. Our results indicate that the pyrethroids tested are more effective in terms of deterrence compared to DDT. Transfluthrin has the highest deterrence when compared to metofluthrin and DDT. It is probably worth noting that the greatest effects of DDT in comparison to those of transfluthrin and metofluthrin are irritancy and toxicity rather than deterrence as indicated in other studies. Further studies are being done to determine the most effective and acceptable format of delivering spatial repellents.

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BEHAVIORAL RESPONSES OF *AEDES AEGYPTI* TO DUET™ AND ITS TWO PYRETHROID COMPONENTS UNDER LABORATORY CONDITIONS

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DUET™ is an insecticide composed of two pyrethroids (1% prallethrin and 5% sumithrin) that is applied as an ultra low volume (ULV) spray to kill adult mosquitoes. It has previously been shown to activate *Culex quinquefasciatus* females in the laboratory resulting in greater mortality. Formulations of DUET™ and its two active pyrethroids were studied to evaluate behavioral responses in 4-8 day-old bloodfed and non-bloodfed ("unfed") *Aedes aegypti* females. Sub-lethal formulations of the pyrethroids and DUET™, and inert ingredients (control) were delivered in a spray cloud of ULV droplets in a wind tunnel and the responses of individual mosquitoes were videotaped. Videotapes were prepared and analyzed during pre-spray, spray and post-spray periods for 80 females using three behavioral analysis programs (*e.g.*, Motus, Ethovision, and Observer) and various behavioral responses (*e.g.*, time to flight, speed of flight, distance flown, duration of flight, and overall flight speed) were measured to determine the impact of the exposure to the different treatments. We found that all three insecticide treatments produced significantly more movement in all groups of females than in the control group. Unfed females moved greater distances when exposed to DUET™ and sumithrin than when they were exposed to prallethrin alone while bloodfed females traveled about the same distance regardless of insecticide treatment. A similar behavioral pattern was observed for overall flight velocity. No distinct pattern of percent time moving was observed regardless of bloodfed status and insecticide exposure. Comparative results of similar studies with *Ae. albopictus* will also be presented and implications of these laboratory results for field applications will be discussed.

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RESISTANCE OF *AEDES AEGYPTI* TO INSECTICIDES IN MARTINIQUE (FRENCH WEST INDIES) AND IMPLICATIONS FOR DENGUE VECTOR CONTROL

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Dengue virus, transmitted by *Aedes aegypti*, is reemerging dramatically in Martinique Island (Caribbean). One of the principal recourses to reduce the transmission remains the fight against the vector by the use of insecticides. Unfortunately, insecticide resistance (metabolic and target site mutation mechanisms) to conventional insecticides (pyrethroid and organophosphate) is strong and widespread among local mosquito populations. The present study was designed to measure and understand the phenotypic impact of resistance on the efficacy of adulticide and larvicide treatments at an operational scale. To assess the impact of pyrethroid resistance on the efficacy of treatments, 3 rounds of applications of deltamethrin and natural pyrethrins were performed with vehicle-mounted thermal foggers in 9 localities of Martinique. Efficacy was assessed by monitoring mortality rates of naturally resistant and laboratory susceptible female mosquitoes placed in sentinel cages. Results showed high mortality rates of susceptible sentinel mosquitoes treated with deltamethrin while resistant mosquitoes exhibited very low mortality. There was no reduction of either larval or adult *Ae. aegypti* population densities after treatments. This suggested a limited efficacy of pyrethroid treatments for reducing the virus transmission during epidemics. Conversely, we showed the potential of using alternative larvicides (spinosad, pyriproxyfen and diflubenzuron) for the control of organophosphate resistant *Ae. aegypti* larvae. Spinosad (naturalyte) and pyriproxyfen (growth regulator) were also used in mixture to measure the residual efficacy of the combination of their different modes of action. Under field conditions, pyriproxyfen and Bti failed to curtail *Ae. aegypti* populations after 4 weeks. Conversely, diflubenzuron and spinosad showed a residual efficacy of 16 weeks suggesting that these chemicals may be promising alternatives to Bti and temephos for controlling insecticide-resistant mosquitoes in Martinique. The mixture remained effective for 18 weeks, showing that the combination of the 2 larvicides acted to increase the residual activity of the treatment. The mixture could preserve the utility of both insecticides in public health programs. This study emphasizes the urgency in the need for further research to provide new tools and innovative strategies to manage insecticide resistance in dengue vectors.

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SUSCEPTIBILITY TEST OF FEMALE *ANOPHELES* MOSQUITOES TO TEN INSECTICIDES FOR INDOOR RESIDUAL SPRAYING (IRS) BASELINE DATA COLLECTION IN NORTHEASTERN NIGERIA

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Malaria is a major public health problem in Nigeria, accounting for about 60% of all outpatient attendances and 30% of all hospital admissions. Several insecticides have been proposed for Indoor Residual Spraying (IRS). However, insecticides vary considerably in their effectiveness against different species and strains of *Anopheles* mosquito.WHO standard insecticide- impregnated papers were used to conduct bioassays in the study area against local populations of *Anopheles* species with a view of selecting the suitable insecticides for IRS. These include: Cyfluthrin (0.15%), DDT (4%), Deltamethrin (0.05%), Lambdacyhalothrin (0.05%), Malathion (5%), Permethrin (0.75%), and Propoxur (0.1%), untreated blank papers were used as control. Two to three days old, female *Anopheles* species, glucose fed, none blood fed, were exclusively used in the bioassay. The result of the knockdown time periods of female *Anopheles* mosquitoes exposed to insecticides-impregnated filter papers after one hour indicated that Alphacypermethrin had the lowest KD_{50} (time taken to knockdown fifty percent of the exposed mosquitoes) values of 4.8 minutes. Relatively moderate KD_{50} values (minutes) were obtained with Propoxur (11.34), Deltamethrin (13.20), Malathion (15.82), Bendiocarb (17.29), Permethrin (18.43), Cyfluthrin (20.28) and Lambdacyhalothrin (23.11). Relatively higher KD_{50} values were obtained with Bifenthrin (27.29) and DDT (32.12) impregnated papers. The results of the 24 h post-exposure mortality indicate that *Anopheles* mosquitoes were susceptible to Alphacypermethrin and Malathion with 100.00% mortality achieved. Suspected cases of resistant were noted with Permethrin and Bendiocarb having mortality values of 96.67% each. On the other hand, cases of resistant were noted with Lambdacyhalothrin (93.33%), Deltamethrin (83.33%), DDT (78.33%) and Cyfluthrin (55.00%). The *Anopheles* species identified during the study were *A. gambae, A. funestus and A. nili.* The public Health significance of these findings is discussed.

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ENTOMOLOGIC BASELINE SURVEILLANCE IN PRELUDE OF INDOORS RESIDUAL SPRAYING IN BAROUELI, MALI

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Baseline data are important for assessing interventions used for disease control. In prelude of implementing IRS in the Baroueli district through PMI/USAID's support, an entomologic baseline study was conducted. Mosquito collections involving pyrethrum spray catches and human landing catches were conducted monthly in three villages from June to October. Bioassays were conducted to assess insecticide resistance in vectors using WHO test kits and five insecticides. Results showed 51% of all mosquitoes collected were *Anopheles* spp, 46% *Culex* spp and 3% *Aedes* spp (n=3399). *Anopheles gambiae s.l.* represented 39% while *An. rufipes* represented 12%. Within *An. gambiae s.l., An. gambiae s.s.* predominated over *An. arabiensis* (89.2% vs 10.8%, n=1403) and the M molecular form predominated over the S molecular form (93.3% vs 6.7% n=1251). Entomological inoculation rates were 0, 2.3, 7.8, 5.8 and 1.4 infective bites per human per month from June to October, respectively. The indoor *vs.* outdoor biting behavior assessment showed a slightly higher mean number of vectors indoors (98.7, SD=109.9) than biting outdoors (85.8, SD=106.2) but the difference was not statistically significant (independent T-test: t=0.187, df=8, p=0.856). Bioassays showed resistance to deltamethrin, permethrin, lambda-cyhalothrin and DDT while bendiocarb, a carbamate, showed a very high efficiency (100% mortality). These data provide valuable updated information on malaria vectors in the district. Of particular interest is the biting/landing behavior. More investigations are needed to better understand the biting behavior as this could be crucial for vector control of malaria.

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SEMI-FIELD EVALUATION OF DURABLE RESIDUAL WALL LINING AS AN ALTERNATIVE TO INDOOR RESIDUAL SPRAYS

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Insecticide-treated nets (ITNs) and indoor residual spraying (IRS) have been widely promoted as primary methods for controlling malaria vectors. However, achieving the desired ITN coverage and usage is challenging and IRS requires regular, repeated insecticide applications. In this study, we are assessing the efficacy of insecticide-treated ZeroVector™ (Vestergaard Frandsen) Durable Lining (DL) as an alternative to IRS for indoor control of *Anopheles* mosquitoes and prevention of malaria. ZeroVector™, a thin, blue sheet of woven shade cloth impregnated with deltamethrin, is being evaluated against 3 species of mosquitoes (*Anopheles quadrimaculatus*, *Culex quinquefasciatus*, and *Aedes aegypti*) and a stable fly (*Stomoxys calcitrans*) under semi-field conditions. Studies are being conducted at the USDA Center for Medical, Agricultural and Veterinary Entomology in Gainesville, FL. The study utilizes 5 wooden huts, with interior measurements of 9'5" x 7'5" x 7'11". Five panels of the DL have been cut, 8'4" L x 7'6" W, and attached vertically to the interior walls of each hut (with a staple gun), with an approximately 2" fold at the ceiling and floor. Untreated panels have been placed in 2 "control" huts and deltamethrin-treated panels have been placed in 3 "treatment" huts. Two types of evaluations are being conducted to determine durable wall liner efficacy. The WHO cone bioassay touch test is being used to evaluate knockdown and mortality caused by the DL against the 3 mosquito species; this test forces the exposed insects to come into contact with the wall lining. Three replicates (consisting of 10 female mosquitoes/cone) of each species were conducted per hut monthly. In the second test, 100 free-flying female mosquitoes of each species and 100 stable flies were released into the huts and able to fly freely and land wherever they chose. One hour after release, a Biogents Sentinel (BGS) trap (baited only with BG-Lure), which had previously been placed in the center of each hut, was activated and allowed to run overnight (ca. 15 hrs). The following day, the traps were collected and all dead mosquitoes were vacuumed from the floor, identified to species, and counted. All remaining flying insects were collected separately in an aspirator and held for 24 hrs and monitored for additional mortality. In the first 6 months of the study, 100% mortality was observed with the cone tests and few mosquitoes or flies were captured in the BGS trap in the treated huts.

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TRANSCRIPTOMICS OF PYRETHROID RESISTANCE IN WILD *ANOPHELES GAMBIAE* MOSQUITOES

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Pyrethroid resistance is one of the most important challenges in malaria vector control. As a consequence, determination of resistance mechanisms and development of cost-effective and reliable resistance monitoring

tools are of primary importance. The understanding of the molecular mechanisms of insecticide resistance in mosquitoes has progressed with technological advancement. Gene amplification-based techniques allowed the identification of two alternative point mutations in the para-type sodium channel gene that leads to knock-down resistance (kdr). Microarray techniques took the analysis of insecticide resistance mechanisms to genome-wide expression profiling and allowed the identification of resistance mechanisms related to transcript expression levels. However, microarrays are limited to the genes spotted on the array and provide relative expression levels, with no sequence variation information. Moreover, additional mechanisms of resistance (i.e. cuticular proteins and/or mitochondrial genes) have been hypothesized but poorly investigated due to the absence of appropriate methodological tools. The recent RNA-seq technology has emerged as an improved method for transcriptome analysis allowing both the absolute transcript quantification and the detection of coding sequence variation. On this basis, we classified field-derived *Anopheles gambiae* mosquitoes into deltamethrin resistant or susceptible on the basis of the standard WHO bioassay test and generated RNA-seq data from both pools. We are analyzing both the difference in transcript expression level and genetic variation in mRNA between RNA-seq libraries from both pools because either gene expression changes or genetic variation may contribute to insecticide resistance. Preliminary analysis detected 433,885 and 364,046 unique SNPs in the susceptible and resistant sample, respectively. Among these SNPs, 140,883 were common in both samples.

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REPORTED ADVERSE AND SERIOUS ADVERSE EVENTS AFTER THE ADMINISTRATION OF INFLUENZA A (H1N1) VACCINE IN CENTRAL GHANA

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The emergence of influenza A (H1N1) virus prompted the development of influenza A (H1N1) monovalent vaccines (2009-H1N1). The use of the vaccine was recommended by the Centers for Disease Control and Prevention (CDC) Advisory Committee on Immunization Practices (ACIP). Adverse events after vaccinations occur but are generally rare. The study was carried out to identify reported adverse and serious adverse events associated with an influenza A (H1N1) vaccine. This cross sectional study was carried out between mid of July 2010 to the 31st of August 2010 in Kintampo North Municipality and Offinso South Municipality in Ghana. The study was carried out as part of the INESS pharmacovigilance study of KHRC. Data were collected from consented participants using questionnaire. Of the 420 forms that were given out to consented participants in the two regions, 379 (90.2%) were returned with completed information related to the Influenza A (H1N1) vaccine. Participants who took the vaccine reported of adverse events such as fever, headache, chills, stomach ache, diarrhoea, pain in the heart and fast heartbeat. 4.4% (16/366) of those who received the vaccine were hospitalized for the adverse event they reported to have experienced after vaccination. Of the 4.4% of the vaccinated participants that were hospitalized, 43.8% (7/16) were males and 56.3% (9/16) were females. There was no difference between the proportions ($p=0.97$) of males and females that were hospitalized after vaccination. In conclusion, Ghana started use of Pandemrix- Influenza A (H1N1) vaccine in June 2010. Symptoms reported ranged from expected to reported death case. This survey of 379 people recorded 16 hospitalizations due to symptoms reported after vaccination. The study was not controlled and therefore could not make claims of whether the serious adverse events were associated with the vaccine. We would want to recommend post-marketing monitoring of adverse events after vaccinations.

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EPIDEMIOLOGICAL TRENDS OF RABIES IN DOMESTIC ANIMALS IN SOUTHERN THAILAND 1994-2008

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Rabies is an acute viral encephalomyelitis that affects wild and domestic mammals. Worldwide, human death due to rabies is approximately 55,000 cases annually. In Thailand, dogs are the main reservoirs and play an important role in rabies transmission. Although recent work indicated that clinical signs and demographics can be used as epidemiological tools for rabies diagnosis, few studies have been published on rabies risk factors in veterinary research. In Thailand, it is thought that rabies prevalence is highest during the hot dry season. People are less aware of rabies outside of this season making it difficult to control rabies year round. This study addresses the relationships between rabies infection and season, time and regions. We identified the risk factors most strongly associated with rabies and the degree to which each factor increased or decreased the odds of rabies infection. We also evaluated spillover assumption of rabies from dogs. Rabies and associated risk factors in dogs, cats and cattle (3,454 animals from 14 southern Thailand provinces submitted between 1994 and 2008) were evaluated using a mixed-effect logistic regression model. The overall prevalence of rabies infection was 48%, with 73%, 51% and 16% of tested cattle, dogs and cats positive for rabies, respectively. There was no seasonal variation in this region so rabies can occur year round in southern Thailand. Among unvaccinated dogs, the odds of rabies were 1.7 times higher than in vaccinated dogs. The odds of rabies were twice as high in dogs with a bite history compared to dogs with no bite history. Dogs less than one year of age had the highest likelihood of rabies. Owned and stray dogs had the same risk of rabies. Aggression was strongly associated with rabies in dogs; thus, most of dog rabies cases in the southern region were the furious form. In cattle, aggression, pharyngeal paralysis, hyperactivity and depression were associated with rabies. The annual fluctuation of the species-specific rabies prevalence suggested a positive correlation between canine and either feline ($r = 0.60$; $P = 0.05$) or bovine rabies ($r = 0.78$; $P = 0.004$). Increased vaccine coverage and public education are needed to reduce the risk of rabies in this population. In highly endemic areas, vaccination in cattle was recommended.

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CLIMATE, LAND USE AND TRAVEL TIMES PREDICT THE SPATIAL ADVANCE OF CASES OF CHIKUNGUNYA DURING AN OUTBREAK IN SOUTHERN THAILAND

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In 2008, Chikungunya re-emerged in Thailand after decades of absence. Cases appeared first in the extreme south of the country and advanced ~300 km over the next 18 months to the middle of the country. The spatial advance of cases appears to have two rates, first advancing slowly from October 2008 to April 2009 then rapidly after April 2009. We hypothesize that climatic variation affected the efficiency of spread in the country, slowing the spatial advance during one period of the year. To try to determine the effect of climate on Chikungunya transmission, we created 4 classes of transmission models, hypothesizing that climate affects; a) the transmission rate from mosquitoes to humans, b) the extrinsic incubation period, c) the fertility rate of mosquitoes and d) the mortality rate of mosquito larvae. We find that models that assume that

temperature and rainfall affects the transmission probability provide the best fit to data from 109 districts in southern Thailand. We find evidence that transmission intensities were high in all parts of southern Thailand at the time of emergence, but the spatial advance was too slow to spread cases throughout the south before changes in climate conditions led to low transmission of Chikungunya. The spatial advance resumed the next season when rainfall totals and mean daily temperature increased. Using a model that explicitly models the spatial transmission process from district to district, we find that a) forestry coverage, b) travel flows of individuals estimated using a gravity model, c) rainfall density and d) temperature and e) driving distance estimated using Google Maps were associated with the speed at which cases advanced.

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MOLECULAR CHARACTERIZATION OF HEMORRHAGIC FEVER VIRUSES CIRCULATING IN NORTHERN GHANA

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Haemorrhagic Fever (HF) viruses are prevalent in West Africa and have led to outbreaks with considerable morbidity and mortality. However, information on prevalence and geographic distribution of these viruses in Ghana is largely lacking. Molecular and serological tools to diagnose typical viral haemorrhagic fevers (VHFs) and research programmes identifying and characterising VHF agents, as well as estimating their public health relevance rarely exist in Ghana. This study seeks to establish prevalence of the causative agents of VHFs and help inform public health policies in Ghana. 8 selected health facilities in Northern Ghana have since July 2008, served as sentinel sites. Patients who meet the case definition are recruited as study subjects. Following informed consent, 5 ml of whole blood is collected by venipuncture and processed onsite into serum. Virus detection and characterization by serological and molecular techniques is then done at the NMIMR, Accra and BNITM, Hamburg, Germany for viral agents associated with VHF. Laboratory analyses have been conducted on 263 serum samples as at January, 2011. Investigations with RT-PCR assays for all the clinical specimens have been negative for HF virus types, Lassa, Crimean Congo, Yellow fever, Dengue, Ebola, Marburg, and Rift Valley. Anti-Lassa fever IgG antibody titers have been recorded for 1 case; one case with both (titers ≥ 1:20); anti-Dengue type-2 IgG (titer ≥ 1:80) and anti-Yellow Fever. Two cases exhibiting specific IgG (titers 1:1280 and 1:1280) and IgM (titers 1:20 and 1:20) against Chikungunya virus respectively were found. Viral RNA were however detected upon differential diagnoses of clinically similar pathogens from the total serum samples for agents including one case of Leptospira *interrogans,* 16 (6.1%) for Hepatitis C, 15 (5.7%) for Hepatitis A, 92 (35.8%) for Hepatitis E and 59 (22.4%) for Hepatitis B viruses. In conclusion, results so far obtained do not indicate a significant presence of VHF viral agents in the Northern regions of Ghana. However, the data generated suggest that viral hepatitis infections, which often share clinical symptoms with viral haemorrhagic fevers, are quite prevalent illustrating the need for differential diagnosis to be implemented.

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CASE-CONTROL STUDY OF RISK FACTORS ASSOCIATED WITH HEPATITIS C

This study was undertaken to identify risk factors for hepatitis C virus (HCV) infection among pregnant women seeking antenatal care in tertiary care hospitals of Karachi, Pakistan. We enrolled 119 cases and 238 controls. Cases were enzyme-linked immunosorbent assay (ELISA III) positive pregnant women for antibodies to HCV; controls were anti-HCV ELISA negative pregnant women. The mean age of study subjects was 26 years (SD 5) ranging from 15 to 50 years. The mean number of pregnancies for cases was 4 (SD 3) and for controls was 3 (SD 2). Among cases an average number of injections in any month was 40%, history of hospitalization was 61% and household contact with jaundice or hepatitis was 35%. In the final multivariable logistic regression model, five or more gestations (aOR = 1.99; 95% CI = 1.08-3.33), ‡1 injection (aOR = 2.33; 95% CI = 1.38-3.91) per month, hospitalization (aOR = 1.78; 95% CI = 1.01-2.99) and household contact with jaundice hepatitis (aOR = 3.32; 95% CI = 1.89-5.83) were independently associated with HCV. Iatrogenic exposure (health care injections, hospitalizations and gestations) is the major risk factor for transmission of HCV among pregnant women. To identify risk factors for hepatitis C virus (HCV) infection among pregnant women seeking antenatal care in tertiary care hospitals of Karachi, Pakistan. We enrolled 119 cases and 238 controls. Cases were enzyme-linked immunosorbent assay (ELISA III) positive pregnant women for antibodies to HCV; controls were anti-HCV ELISA negative pregnant women. results The mean age of study subjects was 26 years (SD 5) ranging from 15 to 50 years. The mean number of pregnancies for cases was 4 (SD 3) and for controls was 3 (SD 2). Among cases an average number of injections in any month was 40%, history of hospitalization was 61% and household contact with jaundice or hepatitis was 35%. In the final multivariable logistic regression model, five or more gestations (aOR = 1.99; 95% CI = 1.08-3.33), 1 injection (aOR = 2.33; 95% CI = 1.38-3.91) per month, hospitalization (aOR = 1.78; 95% CI = 1.01-2.99) and household contact with jaundice hepatitis (aOR = 3.32; 95% CI = 1.89-5.83) were independently associated with HCV. Iatrogenic exposure (health care injections, hospitalizations and gestations) is the major risk factor for transmission of HCV among pregnant women.Iatrogenic exposure (health care injections, hospitalizations and gestations) is the major risk factor for transmission of HCV among pregnant women. Iatrogenic exposure (health care injections, hospitalizations and gestations) is the major risk factor for transmission of HCV among pregnant women.

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THE DETECTION AND MOLECULAR CHARACTERIZATION OF HUMAN ROTAVIRUS G12 GENOTYPES IN THE EASTERN PART OF KENYA

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Globally, rotaviruses (RVs) are the most common cause of severe infantile viral diarrheal disease in infants and children < 5 years of age with ~ 527,000 deaths occurring annually in developing countries. In Africa alone approximately 300,000 young children < 5 years die each year due to RVs. The objective of this surveillance was to determine the epidemiology and the disease burden caused by hospitalization due to rotavirus in children <5 years of age in the Eastern region and to ascertain whether the distribution of rotavirus serotypes in circulation differs from the available rotavirus vaccines strains. Hospital surveillance data for rotavirus infections among children aged < 5years of age was started September 2009 in the Eastern region of Kenya. Cases of acute watery diarrhoea lasting 7 days or less, who are below 5 years of age and had been admitted to the Hospital were enrolled for surveillance. Diarrhoea faecal samples collected from children under 5 years of age with acute gastroenteritis were analyzed by enzyme immunoassays (EIA) and the positive samples genotyped by reverse transcriptase/polymerase chain reaction (RT-PCR) with RV specific primer pairs used for amplification of the VP7 and VP4 gene. From this study G12 was detected for the first time with a G/P combination as G12P [6]. The other genotypes detected were G9 P[4] and G9 P[8]. The common strain detected was G2 P[4]. It was interesting to see that most of the common strains G1 P[8] and G3 P[8] that are already included in the licensed rotavirus vaccines were not identified in this study. In conclusion, rotavirus is an important cause of acute watery diarrhoea in the Eastern region of Kenya among the under five children. The detection of G12 strains from different parts of the world in recent years suggests the possibility of its emergence as an important global genotype. Thus, Monitoring of cocirculating rotavirus strains and detection of emerging strains is important in the context of the availability of rotavirus vaccines. This study has extended our knowledge on the circulating G-genotype circulating in the Eastern region of Kenya.

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PREVALENCE AND CLINICAL OUTCOMES OF CONGENITAL CYTOMEGALOVIRUS INFECTION IN URBAN AND RURAL KENYAN MATERNAL/INFANT COHORTS

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Congenitally acquired cytomegalovirus (CMV) is one of the most prevalent viral congenital illness and amongst the most severe with outcomes including fetal demise, mental retardation, hearing and vision loss. 10% of infants with congenital CMV infection develop mild or severe disease. Estimates of congenital CMV prevalence in industrialized countries range from 0.4-2% while the few studies reporting CMV prevalence in developing countries found >5% prevalence. This study examined congenital CMV prevalence in two maternal/infant Kenyan cohorts, one in an urban setting and one in a rural setting. Congenital CMV is typically diagnosed by culturing infant urine for CMV virus which was not possible in this study. An alternative method of congenital CMV detection is the presence of CMV IgM antibodies in infant cord blood. This study used a high through-put microsphere-based multiplex method to quantify CMV IgM and IgG antibodies in infant cord blood. 521 infant cord blood samples were tested. 77 were found to be positive for CMV IgM antibodies giving an overall prevalence of 12.7%. Significant differences between rural (6.9%) and urban (17.2%) cohort CMV prevalence were found. We will correlate these findings with infant clinical outcomes including hearing and neurocognitive development as well as maternal HIV and malaria co-infections.

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INVESTIGATION OF POTENTIAL CIRCULATION OF HANTAVIRUS AMONG KENYAN WILD RODENTS AND THE IMPLICATIONS FOR PUBLIC HEALTH AND ZOONOSES MONITORING

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Hantaviruses (Family *Bunyaviridae*) have been associated with the human disease Hantavirus pulmonary syndrome (HPS). This virus has been documented to be transmitted to humans through rodent (Family *Murinae*) feces and urine. Evidence of Hantavirus across Africa remains quite scanty, although serological evidence has been published in several countries, including Kenya. To date, two Hantaviruses have been isolated from wild a rodent and a shrew in Guinea, *Sangassou* virus (*Hylomiscua stella*) and *Tanganya* virus *(Crocidura therasae)* respectively. Presence and epidemiology of these viruses across East Africa remain largely unknown. From June 2008 through June 2010, rodents and mammals were trapped across a broad geographic range within Kenya, selected based on varying ecological and climatic conditions, thereby increasing rodent species diversity in the sample population. Total nucleic acid was extracted from lungs of necropsized rodents and reverse transcription Polymerase Chain Reaction used to amplify S and L segment of the Hantavirus genus genome. A total of 392 rodents and small animals consisting of 18 different species of rodents and shrews were trapped. Six samples generated a 494bp fragment with primers designed to amplify the S segment coding for nucleocapsid protein, depictive of Hantavirus presence. Two of these samples were isolated from shrews *(Crocidura species)* and four from *Mastomys species* trapped in semi arid areas of Marigat (Rift valley) and Garissa (North Eastern) Kenya respectively. High throughput pyrosequencing and bioinformatic analysis is underway to characterize the nature, genetic similarity and identity of the PCR positive samples.

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DETECTION OF ALPHA VIRUSES IN MOSQUITOES FROM SEMI ARID AREAS OF KENYA

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Alphaviruses are diverse group principally mosquito-borne RNA viruses that cause diseases in humans worldwide. They include Chikungunya, O'nyong-nyong and Sindbis viruses. They cause febrile illnesses with encephalitis or arthritis. They are of significant public health concern with Venezuelan equine encephalitis virus having potential to be weaponised. To determine the presence and circulation of alphaviruses and the associated vector species responsible for their maintenance and transmission, a surveillance study was undertaken in two semi arid regions of Kenya.Mosquitoes were trapped using CO_2-baited CDC light traps from December 2009 to June 2010 in 6 selected sites in Ijara and Marigat districts during the wet seasons. Mosquitoes were morphologically identified to species, pooled to 25 mosquitoes per pool and homogenized in minimum essential medium using copper beads.The homogenates were clarified by centrifugation at 10,000 rpm and the supernatants inoculated in monolayers of VERO cells in 24 well plates. The cultures were incubated at 37˚C and observed daily for cytopathogenic effects (CPE). Cultures showing CPE were harvested and viruses identified by RT-PCR and sequencing.Over 92,000 mosquitoes were collected, identified into 37 species and pooled into 4,382 pools. Eleven NDUV isolates were obtained from pools of *Aedes mcintoshi* (7), *Ae. ochraceus* (1) and *Ae. tricholabis* (2) all collected from Ijara and *An. pharoensis* (1) from Marigat. SFV was isolated from *Ae. ochraceus* (3) and *Ae. tricholabis* (2) from Ijara and one isolate of SINV from *Culex antenattus* from Marigat.This study shows that SFV, SINV and NDUV are circulating among mosquito species in the two semi arid regions of Kenya and could account for some of the febrile illnesses of unknown etiology observed in these areas. NDUV was found in both sites. SFV was detected in Ijara while SINV was found in Marigat. Human surveys are being conducted to establish the actual involvement of human population in the circulation of the viruses. Control strategies to prevent alphavirus transmission should target the three mosquito genera.

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DIFFICULTIES IN ACCESSING SPECIALIZED MEDICAL CARE BY ENCEPHALITIS CASES DURING A NIPAH VIRUS OUTBREAK IN BANGLADESH

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Nipah virus (NiV) causes fatal encephalitis in humans. Previous outbreak investigations in Bangladesh have identified drinking raw date palm sap contaminated by Pteropus bats, the reservoir host of NiV, and person-to-person transmission as the major risk factors for NiV transmission. Reluctance of hospital health workers to provide hands-on care to NiV cases has been reported. During December 2010 - February 2011, we investigated an outbreak of NiV infection in Bangladesh to understand the risk factors for acquiring the disease and to explore the medical care received by NiV cases. We collected clinical and exposure history of the cases. We conducted a case control study to identify risk factors; 4 neighborhood controls were selected for each case. We explored

the medical care received by NiV cases at hospitals through in-depth interviews, informal and group discussions with family members and health care providers. We visited isolation wards in two hospitals to observe the care received by admitted cases. We identified 31 Nipah cases, of which 18 were Nipah IgM antibody positive. All cases died. In bivariate analysis, drinking raw date palm sap was the only risk factor for NiV infection (OR 17; 95% CI 4-70). Among the 31 cases, 30 (97%) were hospitalized. Twenty four cases were directly admitted or referred to tertiary hospitals for specialized care. Seven were transferred twice or more from tertiary hospitals. Family members of cases and a health care provider reported that some hospitals refused admission or transferred patients to a different facility if they were from a Nipah affected area or had a history of consuming raw date palm sap. Family members also reported unwillingness of providers to attend admitted NiV cases. One case, who had been weaned from mechanical ventilation, was forced to leave the hospital after laboratory confirmation of NiV infection. Subsequently, she was refused admission to another tertiary hospital. In isolation wards, patients were admitted without proper evaluation, NiV and non NiV cases were kept close to each other and had to share oxygen masks. Bangladeshi people should avoid drinking raw date palm sap to prevent transmission of NiV infection. Hospitals should develop strategies to triage and treat patients with diseases that transmit person-to-person, organize training for hospital staff on providing appropriate care to these patients and ensure implementation of infection control practices.

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COMPLEX SEASONAL FLUCTUATION IN ARBOVIRAL ACTIVITY IN TROPICAL AND TEMPERATE AUSTRALIA

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Mosquito-borne diseases typically exhibit strong seasonal patterns with a series of outbreaks followed by low endemic levels outside the typical season. Occasionally, the period of low incidence contains secondary peaks of smaller magnitude that are difficult to detect using traditional methods and aggregated monthly data across years. More precise methods that capture the variability in incidence across a series of annual cycles may enhance the design of early warning systems for such diseases. To illustrate, we applied a Poisson harmonic regression model with polynomial components to capture non-linear trends in the incidence of three arboviruses - Barmah Forest virus (BFV), Ross River virus (RRV), and dengue virus (DENV) - as reported to Australia's National Notifiable Diseases Surveillance System from 1991 to 2010. For each infection, we estimated major seasonality characteristics - peak timing and amplitude - and their confidence intervals using recently introduced delta-methods. Strong annual periodic fluctuations were observed for BFV and RRV, with increased seasonal activity (defined as incidence >1SD above the annual mean) occurring within a narrow time interval (generally February through April). Nevertheless, the onset of increased seasonal activity varied by as much as 2 months between years. Further, clearly defined outbreaks (incidence >2SD above the annual mean) were only noted in certain years. Secondary peaks, occurring in October through December, were an emerging phenomenon, appearing in the latter half of the time series for both BFV and RRV. In contrast, the seasonal pattern for DENV comprised floating primary and secondary peaks that varied considerably in terms of timing and amplitude between years. We also detected very specific oscillations in DENV every 5-7 years. These findings suggest that triggering environmental or other factors may vary from year to year, and may be changing over time. The findings illustrate how application of advanced analytical tools can enrich our understanding of the complex seasonal fluctuations of mosquito-borne diseases. Future work will explore the extent to which these predictive models can be enhanced by incorporating local environmental conditions, both temporal (e.g. precipitation, temperature) and spatial (e.g. proximity to estuarine habitat), using remote sensing and meteorological data.

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DEVELOPMENT OF A REVERSE GENETIC SYSTEM TO STUDY THE IMPACT OF THE P GENE PRODUCTS ON THE ENDOTHELIAL CELL INNATE ANTIVIRAL RESPONSE AGAINST NIPAH VIRUS

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The henipaviruses, Nipah virus (NiV) and Hendra virus (HeV), are highly pathogenic zoonotic paraymyxoviruses that cause fatal encephalitis in up to 75% of infected humans. Like other paramyxoviruses, henipaviruses employ a process of co-transcriptional mRNA editing during transcription of the phosphoprotein (P) gene to generate additional mRNAs encoding the V and W proteins. The C protein is translated from the P mRNA, but in an alternate reading frame. Sequence analysis of multiple, cloned mRNAs showed that the mRNA editing frequencies of the P genes of the henipaviruses are higher than those reported for other paramyxoviruses. Mouse antisera against synthetic peptides from the P, V, W, and C proteins of NiV were generated to study their expression in infected cells. All proteins were detected in both infected cells and in purified virions. In infected Vero cells, the W protein was detected in the nucleus while P, V, and C were found in the cytoplasm. Since endothelial cells and neurons are important targets for NiV pathogenesis in humans, we measured viral replication and innate immune responses in NiV infected primary endothelial cell types and one neuronal cell line. NiV infected endothelial cells generated a functional IFN-β response, which correlated with the unexpected localization of the NiV W protein to the cytoplasm. There was no antiviral response detected in infected neuronal cells. NiV infection of endothelial cells induced a significant increase of inflammatory chemokines secreted into the cellular supernatant, and these supernatants induced a corresponding increase in monocyte and T-lymphocyte chemotaxis. Our results suggest that the induction of pro-inflammatory chemokines in NiV infected primary endothelial cells *in vitro* is consistent with the prominent vasculitis observed in infections, and provide initial molecular insights into the pathogenesis of NiV in physiologically relevant cell types. We have now developed a reverse genetic system to study the individual roles of the NiV P gene products on the endothelial cell innate immune response.

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CROSS PROTECTIVE IMMUNITY AGAINST O`NYONG-NYONG VIRUS AFFORDED BY A NOVEL RECOMBINANT CHIKUNGUNYA VACCINE

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Emerging mosquito-borne alphavirus infections caused by chikungunya virus (CHIKV) or O`nyong-nyong virus (ONNV) are responsible for sporadic and sometimes large explosive epidemics. In particular, ONNV that is transmitted by anopheles mosquitoes has been the cause of a major epidemic in Africa which involved at least 2 million patients between 1959 to 1962. For decades, CHIKV has been an important etiologic agent of

human disease in Africa and Asia. The virus recently reemerged in the Indian Ocean islands, India and Southeast Asia causing several million cases of severe and often chronic arthralgia. Recently, we developed a candidate CHIKV vaccine by employing a genetic attenuation mechanism. The internal ribosome entry site (IRES) from encephalomyocarditis virus was used to replace the sub-genomic promoter in a cDNA CHIKV clone, thus altering the level and host-specificity of structural protein gene expression. The testing of vaccine in both normal outbred mice and interferon response-defective (A129) mice demonstrated that it is highly attenuated, immunogenic and efficacious after a single dose. Furthermore, the genetically attenuated vaccine virus was incapable of replicating in mosquito cells or infecting mosquitoes *in vivo*. In this study we sought to investigate the capacity of the CHIK/IRES vaccine to induce cross protective immunity against the closely related ONNV. Our studies demonstrated that the CHIKV/IRES candidate vaccine elicited strong cross neutralizing antibodies against ONNV and conferred protection against challenge with this virus after a single administration. Moreover, the role of antibodies in protection was established by demonstrating their efficacy in two models; i) CHIKV/IRES immune A129 dams transfer antibodies to their offspring that protect against ONNV challenge, and ii) anti-CHIKV/IRES antibodies confer protection in AG129 mice against ONNV independently of a functional IFN response.

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TICK-BORNE ENCEPHALITIS VIRUS ANTIGEN IN TICKS AND MILK SAMPLES IN SOUTHERN AREAS OF THE REPUBLIC OF KAZAKHSTAN

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Tick-borne encephalitis is a serious human disease, whose incidence and geographic location is expanding in several areas of the world including regions in Central Asia. Tick-borne encephalitis virus (TBEV), a flavivirus, is harbored by ticks in Kazakhstan and evidence also shows high levels of seroconversion in human sera. Using an antigen-capture ELISA, we analyzed ticks and milk from cows and sheep for TBEV in one endemic and three non-endemic territories of Kazakhstan. In the Almaty oblast, where TBEV is endemic, we tested samples from 1,295 ticks that were collected and pooled into 28 groups based on size and maturity. TBEV antigen was found in 3 of 19 groups of 1056 *Dermacentor marginatus* ticks, 0 of 5 groups of 235 *Haemaphysalis punctate* ticks, and 0 of 4 individual *I. persulcatus* ticks. In the same area, we examined 45 milk samples (15 sheep and 30 cows) collected from local individuals. TBEV antigen was detected in 3 sheep milk samples and 0 cow milk samples. We also conducted testing in three oblasts considered non-endemic: Kyzylorda, Zhambyl, and South Kazakhstan. In Kyzylorda, TBEV antigen was found in 6 of 142 pooled groups of 3,500 *D. marginatus* ticks. In Zhambyl, TBEV antigen was also found in 6 of 10 pooled groups of 250 *D. niveus* ticks. In South Kazakhstan, TBEV antigen was found in 17 of 40 pooled groups of 1,338 *H. asiaticum* ticks. Our data clearly show that ticks infected with TBEV are present not only in areas previously considered to be endemic (e.g., Almaty oblast), but also in the additional oblasts of Kyzylorda, Zhambyl and South Kazakhstan where TBE disease is not registered by public health authorities. Also, we show that in the endemic Almaty oblast, risk of infection with TBEV may take place not just through an infected tick, but also by consumption of infected milk or milk products.

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FETAL EFFECTS OF INFLUENZA IMMUNIZATION IN PREGNANCY

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We report the association of maternal antenatal influenza immunization with fetal and neonatal outcomes. The Mother's Gift project was a blinded, randomized trial of 340 pregnant urban Bangladeshi women who were randomized to receive either inactivated influenza vaccine or pneumococcal vaccine (control group). Gestational age, proportions of small for gestational age (SGA) infants, and mean birth weights were compared in 327 neonates.There was a reduction in % SGA infants from 38% in influenza vaccinees to 28% in controls ($p = 0.05$) and a trend of increased birth weights in flu vaccine recipients ($p = 0.09$), with no differences in mean gestational ages. Influenza virus did not circulate from August 2004 through January 2005, and the study groups were similar in the incidence of respiratory illness with fever (RIF) ($p=0.99$); during this interval, % SGA infants and mean birth weights were similar between the study groups. In contrast, during the interval of influenza virus circulation from February to June 2005 there was a 49% reduction of RIF episodes in the influenza vaccine group ($p=0.0003$). During the interval of influenza circulation, the % SGA infants was substantially decreased in the influenza vaccine group to 29% versus 44% in controls ($p=0.03$). Similarly, the mean birth weight of infants of influenza vaccinees was 3,178gm vs. 2,978gm in controls ($p=0.03$). In conclusion, influenza immunization of pregnant women substantially reduces the proportion of SGA infants and increases mean birth weights in this South Asian setting. These data suggest that influenza infections in pregnancy adversely effect fetal development, and further studies are needed to assess this unique observation.

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THE BURDEN OF PEDIATRIC DIARRHEA: PERCEPTIONS OF COST AMONG BOLIVIAN CAREGIVERS

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Bolivia has high rates of diarrhea related child morbidity and mortality. While child diarrhea is known to be costly to the Bolivian state, Bolivian caregivers benefit from universal insurance for their children and are believed to have minimal expenses associated with diarrhea. The study goal was to characterize caregiver costs and cost perceptions associated with seeking treatment for pediatric gastroenteritis in Bolivia. From 2007 to 2009, researchers interviewed 1101 caregivers of pediatric patients (<5 years of age) seeking treatment for diarrheal illness in five healthcare settings, in three geographic regions, and participating in a diarrheal surveillance program throughout Bolivia. Caregivers were surveyed on child demographics, clinical symptoms, direct (e.g., medication, consult fees) and indirect (e.g., lost wages) costs, and perceived economic burden of the child's diarrheal illness. Patient populations were similar across hospitals in terms of gender, age, appointment type, and duration of illness, while familial income varied when stratified on appointment type. Direct and indirect costs to families were significantly higher for inpatients as compared to outpatients ($p < 0.01$). Overall, 74% of caregivers reported that the cost of treatment affected their family economy, and this proportion differed significantly among hospitals ($p < 0.0001$) and by cost burden (cost of treatment as a percentage of family income; $p < 0.0001$). Logistic regression indicated significant positive associations of cost perception with cost burden (OR 20.83 95% CI [4.39 - 98.98]) and appointment type (outpatient vs. inpatient, OR 2.06, 95% CI [1.22 -3.49]). Diarrhea related costs were a large burden on Bolivian families, and those with a high cost burden were most likely to perceive these

costs as posing economic hardship. While overall costs were higher for hospitalized patients as compared to outpatients, perceptions of cost were higher among caregivers of outpatients. Families who perceive outpatient treatment as costly may delay care, possibly resulting in the need for more expensive inpatient care and potentially poorer health outcomes.

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PARTICIPATORY MAPPING AS A COMPONENT OF OPERATIONAL MALARIA VECTOR CONTROL IN TANZANIA

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Global efforts to tackle malaria have gained unprecedented momentum. However, in order to move towards the ambitious goal of eliminating and eventually eradicating malaria, existing tools must be improved and new tools developed. The City of Dar es Salaam, Tanzania, is home to the first operational community-based larviciding programme targeting malaria vectors in modern Africa. In an attempt to optimize the accuracy of the application of larvicides, a participatory mapping and monitoring approach has been introduced in 2005 that includes (1) community-based development of sketch maps of the target areas, and (2) verification of the sketch maps using laminated aerial photographs in the field which are later digitized and analyzed using Geographical Information Systems (GIS). The participatory mapping approach developed enables gap-free coverage of targeted areas with mosquito larval habitat control, and more equal distribution of the workload of field staff. The procedure has been tested, validated and successfully applied for five years within the operational larviciding programme. During the same period, the mapping coverage has been scaled up from 56 km^2 to an area of about eight times that size, thus covering the urban area of Dar es Salaam. The Government of Tanzania is currently scaling up the larviciding programme to the whole city region, using the map data and mapping procedure as a basis. The procedure is simple, straightforward, replicable and at relatively low cost. It requires only minimal technical skills and equipment. In the case of Dar es Salaam, the resulting database provides a spatial resolution of administrative boundaries that is almost 50 times higher than that of previously available data. This level of detail can be very useful for a wide range of other purposes rather than merely malaria control, for example implementation of council programmes in a variety of sectors and spatially-explicit analyses for research and evaluation purposes.

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DISTRICT-BASED HOUSEHOLD SURVEY DATA AND ASSOCIATED BIOMARKERS IN INDOOR RESIDUAL SPRAYING (IRS) AND NON-IRS DISTRICTS IN NORTHERN UGANDA

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Indoor residual spraying (IRS) with insecticides is a primary intervention to reduce malaria transmission. In highly malaria-endemic Northern Uganda, selected districts have been sprayed since 2007 with DDT and pyrethroids, before localized political opposition to DDT and documented resistance to pyrethroids prompted a shift to carbamates in 2010. Data from a household survey and associated biomarker collection in late 2010 in three contiguous districts of Northern Uganda were used to compare one non-sprayed district (Lira) with two IRS districts (Apac, sprayed once with carbamates in 2010 after one round each of DDT (2008) and pyrethroids (early 2010) and Pader, which received two rounds of carbamate spraying in 2010, following four rounds of pyrethroids (2007-2009)). District-level anemia and parasitemia prevalence estimates from a total of 1,773 children less than five years of age were calculated from the two-stage, cluster sample survey, using sampling weights and accounting for clustering. Parasitemia levels were significantly lower in both IRS districts compared to the non-sprayed district. In Apac, 37.2% of children had positive malaria blood smears, compared to 50.1% of children in non-sprayed Lira district, $p<0.01$. Parasitemia prevalence was lowest in Pader (16.9%, $p<0.001$ compared to both Apac and Lira), which had been sprayed twice with carbamates in 2010. Anemia (hemoglobin<11g/dL) was less common in Apac (38.4%) and Pader (36.9%), compared to Lira (53.0%), $p<0.001$. Bednet use by children was significantly higher in the IRS districts (69.6% in Apac and 64.6% in Pader) than in Lira (49.5%), but there were no significant differences between the districts in terms of food security or distance to the nearest health facility. These results indicate lower malaria burdens, according to biomarkers, in IRS districts compared to non-sprayed districts in Northern Uganda. Additional research is needed to better define causal relationships between IRS schedules and formulations and reductions in malaria indicators in areas of high transmission intensity.

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SPATIAL DISTRIBUTION OF BEDNET COVERAGE UNDER ROUTINE DISTRIBUTION THROUGH THE PUBLIC HEALTH SECTOR IN A RURAL DISTRICT IN KENYA

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Insecticide-treated nets (ITNs) are one of the most important and cost-effective tools for malaria control. Maximizing individual and community benefit from ITNs requires high population-based coverage. Several mechanisms are used to distribute ITNs, including health facility-based, targeted distribution to high-risk groups; community-based mass distribution; social marketing with or without private sector subsidies; and integrating ITN delivery with other public health interventions. Here we use data from a population-based census of more than 44,000 households to examine the extent of coverage with bednets in a district in western Kenya where the primary mechanism for distribution is to pregnant women and infants who attend antenatal and immunization clinics. We use both multivariable logistic regression and spatial techniques to explore the relationship between household bednet ownership and sociodemographic and geographic variables. We show that only 21% of households own any bednets, far lower than the national average of 60%. Ownership in households that include a member of a targeted group, either a pregnant mother or child under-5, was slightly higher; 24% and 25%, respectively, compared to 17% in households with neither. Pregnant women attending antenatal clinic were not more likely to own a bednet than pregnant women not attending antenatal clinic. We also show that coverage is spatially heterogeneous with less than 2% of the population residing in zones with adequate coverage to experience indirect effects of ITN protection. Wealth indicators such as land ownership and animal ownership had a larger effect on bednet ownership in urban than rural areas. The type of nearest facility (hospital, health centre or dispensary) was more important than the absolute distance to the facility in predicting bednet ownership, although both were significant in the multivariable regression model.

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FROM INTERVENTION TO IMPACT: MODELLING THE POTENTIAL MORTALITY IMPACT ACHIEVABLE BY DIFFERENT LONG-LASTING INSECTICIDE-TREATED NETS (LLIN) DELIVERY STRATEGIES

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The current target of universal access to long-lasting insecticide-treated nets (LLINs) is 80% coverage, with a goal of reducing malaria deaths by 75% or more by 2015. So far, mass distribution campaigns have been the main channel for large-scale delivery of LLINs, and more recently WHO has recommended that equal priority should be given to delivery via routine antenatal care (ANC) and immunisation systems (EPI) to target pregnant women and children from birth. These various channels of LLIN delivery are targeted to children of different ages. Since risk of mortality varies with child age, and LLIN effectiveness declines with net age, we hypothesise that the age at which a child receives a new LLIN, and therefore the delivery channel, is important in optimising the health impact of that net. We developed a dynamic mathematical model of delivery and impact of LLINs among children under five years of age and their household members, incorporating data on age-specific malaria death rates at different endemicities, net efficacy over time and net use by household structure. LLINs are assumed to be discarded at a constant rate after delivery. Our analysis found that a universal campaign giving 2 LLINs per household every 3 years with 80% coverage at delivery in a high transmission setting would achieve an annual average 23% reduction in under-five malaria mortality. If supplemented by an ANC distribution system giving one LLIN per birth with 80% of eligible women receiving a net, the mortality reduction achieved is 1.4 times higher, with very little additional redundancy in impact per LLIN, reflecting that children born in the years between distribution campaigns would otherwise have access to old nets or no nets at an age of high risk. This advantage holds if campaign delivery is targeted to under-fives giving one LLIN per child or if malaria endemicity is medium-to-low. Our results indicate that LLIN delivery policies must take into account the age of greatest malaria risk. Strong emphasis should be placed on supporting routine delivery of LLINs to young children as well as campaigns.

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SURVEILLANCE OF VECTOR POPULATIONS AND MALARIA TRANSMISSION DURING AN EL NIÑO IN THE WESTERN KENYA HIGHLANDS: OPPORTUNITIES FOR EARLY DETECTION OF MALARIA HYPER-TRANSMISSION

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Vector control in the highlands of western Kenya has resulted in significant reduction of malaria transmission and a change in the vectorial system. Climate variability as a result of events such as the El Nino increases the suitability of malaria transmission in the highlands. Surveillance and monitoring of transmission is an important component of early risk identification and management. However below certain disease transmission thresholds the traditional tools for surveillance such as the entomological inoculations rates may become insensitive. We carried out a study to determine the usefulness of a rapid diagnostic kit based on the prevalence of *Plasmodium falciparum* circumsporozoite surface protein and merozoite surface protein antibodies in humans for early detection of transmission surges in the western Kenya highlands. Indoor resting adult malaria vectors were collected in Western Kenya highlands in four selected villages categorized into two valley systems, the U shaped (Iguhu and Emutete) and the V shaped valleys (Marani and Fort Ternan) for eight months. Members of the *Anopheles gambiae* complex were identified by PCR. Blood samples were collected from children 6-15 years old and exposure to malaria was tested using Circum-sporozoite protein and Merozoite surface protein immunchromatographic rapid diagnostic test kit. Sporozoite ELISA was conducted for detection of circum-sporozoite protein. Among the four villages studied an upsurge in antibody levels was first observed in October 2009. *P. falciparum* sporozoites were then first observed in December 2009 at Iguhu village and February 2010 at Emutete. Despite an upsurge in antibody levels in Marani and Fort Ternan no sporozoites were detected throughout the eight month study period. The antibody based assay had much earlier transmission detection ability than the sporozoite based assay. Prior to 2002, no *An. arabiensis* had been reported in the western Kenya Highlands. In this study the proportion of *An. arabiensis* among *An. gambiae* s.l. ranged from 2.9-66.7% indicating a rearrangement of the species complex. This is an adaptation to insecticide interventions and climate change. The changing malaria transmission rates in the western Kenya highlands will lead to more unstable transmission, decreased immunity and a high vulnerability to epidemics unless surveillance tools are improved and effective vector control is sustained.

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IMPACT OF ARTEMETHER-LUMEFANTRINE (AL) AS FIRST LINE TREATMENT POLICY ON MALARIA TRANSMISSION AND UNDER FIVE MORTALITY IN A RURAL AREA WITH HIGH INSECTICIDE-TREATED NET (ITN) COVERAGE IN TANZANIA

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Wide use of artemisinin-based combination therapy (ACT) in addition to vector control measures is recommended in the fight against malaria. The ALIVE [Artemether-Lumefantrine In Vulnerable patients: Exploring health impact] project, assessed the impact of AL as first line treatment for uncomplicated malaria on transmission and <5yrs (U5) child mortality in Tanzania. Parasite prevalence was obtained by repeated cross-sectional surveys in two rural districts during two separate periods of first line anti-malarial therapy (2004-2006: sulfadoxine-pyrimethamine [SP], and 2008-2010: AL). Mortality rates were obtained using a demographic surveillance system. Changes in community malaria parasitaemia and U5 mortality between both periods were compared taking into account the contribution of malaria interventions and contextual factors such as rainfall and rice yields using linear and Poisson regression models. Overall, asymptomatic parasite prevalence (%) progressively declined from 25.0 in 2004 to 3.9 in 2010. A 10% increase in community net ownership was associated with a 4.6% reduction in parasitaemia (95% CI= -8.0% to -1.2%). Mean U5 mortality rate decreased by 33% over the entire period, from 27.0 per 1,000 person years in 2005 to 17 in 2009. The introduction of AL was associated with an 11% decrease in U5 mortality when adjusted for other key malaria interventions and contextual factors (IRR= 0.89; 95% CI= 0.79-1.0). One unit (ton of rice/ha) annual increase in rice yields, was associated with a 36% reduction in annual U5 mortality (IRR= 0.64; 95% CI= 0.54-0.75). On the contrary, ITN coverage was not responsible for significant reduction in U5 mortality. ACT implementation with AL in Tanzania together with other major malaria control programmes was associated with a considerable decline in malaria and U5 mortality. Food security with other key malaria interventions is crucial to support malaria control hence elimination.

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ANOPHELES SALIVARY GSG6-P1 PEPTIDE, AN IMMUNO-EPIDEMIOLOGICAL BIOMARKER FOR PERTINENT EVALUATION OF EXPOSURE HETEROGENEITY TO *ANOPHELES* BITES AND EFFICIENCY OF MALARIA VECTOR CONTROL STRATEGIES IN URBAN SETTINGS OF AFRICA

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Urban malaria is becoming a serious public health problem in Africa. Classical entomological and parasitological methods to assess malaria risk and vector control strategies (MVCS) present considerable limitations in urban context. A simple and highly sensitive tool is needed for a precise evaluation. Human antibody (Ab) responses to the specific *Anopheles* salivary gSG6-P1 peptide was describe to be a pertinent biomarker evaluating human exposure to *Anopheles* bites and the efficacy of MVCS. The aim of this work was to validate the gSG6-P1 as an epidemiological indicator evaluating malaria risk heterogeneity and MVCS efficiency used by urban populations of Dakar (Senegal), one of the biggest cities in West Africa. One cross-sectional study (October-December 2008) concerning 3,000 randomly selected children and adults (1,435 households) living in 45 districts of Dakar and its suburbs was performed from October to December 2008. Results show considerable variations in individual anti-gSG6-P1 IgG levels between and within districts. In spite of this inter-individual heterogeneity, the level of specific IgG and the percentage of immune responders differed significantly between districts. According to anti-gSG6-P1 IgG results, three groups of exposure's intensity to *Anopheles* vectors (low, medium and high) were constituted. More significant differences between exposure groups were obtained using the anti-gSG6-P1 IgG tool ($P < 0.0001$) compared to results of exposure to *An. gambiae* bites evaluated by classical entomological method. In addition, multivariate analysis shows that specific IgG responses was age-dependant and significantly lower for individuals who especially used particular MVCS such as bed-nets and spray bombs. Specific IgG responses to gSG6-P1 peptide could represent a new alternative tool to evaluate the heterogeneity of exposure level to bites, malaria transmission risks and used MVCS efficiency in urban settings, at the population and individual levels.

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CONFIGURATION OF THE BG-SENTINELTM (BGS) MOSQUITO TRAP FOR AN *AEDES AEGYPTI* (*DIPTERA:CULICIDAE*) "PUSH-PULL" CONTROL STRATEGY AT THE HOUSEHOLD LEVEL

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Our previous studies have quantified recapture rates of *Aedes aegypti* using varying BioGents Sentinel™ (BGS) trap densities and mosquito release numbers under screen house (140m^3) conditions. Further studies determined the potential effects of exposure to spatial repellent chemicals on host-seeking behaviors of female *Ae. aegypti* mosquitoes and subsequent BGS trapping success. Optimization of the physical parameters - location and distance of traps from huts- that may affect BGS efficacy were also performed. We report here on validating the findings from these experimental conditions (i.e., screen house and experimental huts) in a local village environment in Thailand to determine correlates between the two scenarios. This current work is also aimed at describing the optimum BGS conditions for a planned pilot evaluation of a push-pull system (combining both a spatial repellent and the BGS trap) in a selected community in Chiangmai, Thailand. Results show that the use of BGS traps under the predetermined experimentally optimized conditions, to include distance, number and location of the BGS trap at a single household, functioned to capture *Ae. aegypti* adults despite the presence of competing resting sites and hosts under real-home settings. Findings will elucidate the full potential of the BGS trap as the pull component, as well as the challenges in implementing the complete system, under a typical endemic environment in support of our larger proof-of-concept research program.

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SPATIAL REPELLENCY RESPONSES OBSERVED IN *AEDES AEGYPTI* TO REDUCED DOSES AND SURFACE AREA COVERAGE OF CHEMICAL COMPOUNDS IN WESTERN THAILAND

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Current control strategies for adult *Aedes aegypti*, a vector of dengue, are focused on toxic actions of chemical compounds but increasing case numbers and distribution of dengue fever world-wide highlight that other approaches are warranted. Our larger research program is focused on evaluating sub-lethal chemical approaches in a Push-Pull system to reduce *Ae. aegypti* densities inside homes using minimal chemical dose and treatment coverage of spatial repellents. Here we report on behavioral responses of female *Ae. aegypti* in response to transfluthrin, one of the promising candidate spatial repellent compounds. Insecticide treated material strips of different surface area coverage and doses were placed on the interior walls of experimental huts at our Thailand field site. Entry movement patterns, knock down and toxicity rates of mosquito test populations were quantified and compared to a matched control. Our finding revealed that transfluthrin produced a strong insecticidal action at field application rate using high surface area coverage while rates below the field application rate applied at minimal coverage also significantly reduced the densities of *Ae. aegypti* entering into the huts but without toxic effects. This suggests that spatial repellency can reduce human-vector contact inside homes while minimizing insecticide resistance selection pressure, a key factor in future insecticide management. Data from this study will be used in modeling efforts to determine potential disease impact on dengue transmission using spatial repellency and to guide selection of the repellent compound treatment scheme for a Push-Pull pilot study.

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POOLED SEQUENCING AND ARRAY HYBRIDIZATION TO IDENTIFY INSECTICIDE RESISTANCE GENES IN A MALARIA MOSQUITO VECTOR

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The *Anopheles gambiae* mosquito is the principal vector of malaria in sub Saharan Africa, where malaria-induced mortality is most severe. The use of insecticides to control disease transmission has been demonstrated to be highly effective. Resistance to insecticides is increasingly common in *A. gambiae*, however, and threatens to undermine the efficacy of malaria control programs. To learn more about the genetic basis of insecticide resistance in this vector, we undertook both pooled Illumina sequencing and pooled SNP array hybridization of mosquitoes typed as resistant or sensitive to lambda-cyhalothrin, a pyrethroid insecticide commonly used to treat bed nets. A total of 40 pools were generated using an average of 15 sibling females with consistent phenotypes, which were reared from eggs laid by wild-caught Ugandan mosquitoes. This pooling strategy was devised to hedge against the extremely short linkage disequilibrium in the *A. gambiae* genome and improve our power to observe phenotype-associated SNPs in the array analysis. Comparison of the pools resulted in a surprisingly polygenic profile of the insecticide resistance phenotype in Ugandan *A. gambiae* mosquitoes. Analysis yielded several dozen genomic regions significantly associated with permethrin resistance, in both the array data and sequencing results. Candidate resistance loci include probable insecticide targets (voltage gated ion transporters) as well as genes most likely involved in metabolic resistance mechanisms. Despite the high density of markers on the SNP array (400K total SNPs/1 per 600 bp), most hits on the array were based on single markers due to the extremely short LD in *A. gambiae* mosquito populations. This makes sequence data very useful in detecting markers not associated with recent selective sweeps. Given the prevalence and volatility of insecticide resistance in *A. gambiae* populations, discovery and monitoring of resistance markers through sequencing or pooled array hybridization could be of great importance in the strategic implementation of future malaria control programs.

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ANOPHELES GAMBIAE-SELECTIVE AND RESISTANCE-BREAKING ACETYLCHOLINESTERASE INHIBITORS FOR MALARIA CONTROL

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Recent advances in malaria control in sub-Saharan Africa are threatened by growing resistance to pyrethroids, the class of insecticides used on current generation insecticide-treated nets (ITNs). To address this problem, we seek to develop acetylcholinesterase (AChE) inhibitors that are 1) safe to humans and 2) possess low cross-resistance to *Anopheles gambiae* carrying the G119S AChE resistance mutation. Agricultural carbamate insecticides show very low selectivity for inhibition of *An. gambiae* AChE over human AChE, but we found that appropriate structural modification of these compounds can confer up to 500-fold selectivity. Such levels of selectivity could significantly reduce human toxicity of carbamates. Reasoning that the G119S resistance mutation reduces the volume of the AChE active site, we explored carbamate inhibitors in which the typical 6-membered aromatic ring was replaced with a smaller core. We will disclose a series of pyrazole-based carbamates that show good contact toxicity to AKRON strain *An. gambiae*, which carries both the G119S AChE mutation and the L1014F kdr mutation of the voltage-gated sodium ion channel. Kinetic studies of the inhibition of WT and G119S *An. gambiae* AChE demonstrate that greater potency against the G119S enzyme accompanies their observed higher toxicity relative to standard carbamate insecticides.

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DYNAMICS OF INSECTICIDE RESISTANCE IN *ANOPHELES GAMBIAE* S.L. ACCORDING TO COTTON CULTIVATION SCHEMES IN BURKINA FASO, WEST AFRICA

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Resistance to insecticides in the mosquito, *Anopheles gambiae* is a major threat to sustainable malaria vector control in Africa. Here, we present new data from Burkina Faso, where longitudinal and cross-sectional surveys were to conducted to i) explore the level of resistance to the four classes of insecticides available for public health and ii) monitor the frequency of the L1014F and L1014S kdr mutations in field *An. gambiae* populations throughout the country. Our sampling sites were chosen to belong to one of three ecological settings including: areas of extensive industrial cotton cultivation with high levels of insecticide usage for crop treatment, areas of limited experimental parcels of biological cotton cultivation with no insecticide usage, and areas of transgenic cotton cultivation with low insecticide usage. Mosquitoes were collected as larvae during the spray and non-spray periods in 2008 and 2009. They were brought back to the laboratory and reared to adults. Adult susceptibility tests were carried out using standard WHO protocols: susceptibility to DDT, permethrin, deltamethrin, Chlorpyriphos Methyl (CM) and bendiocarb was assessed. Test specimens were further identified to species and molecular form and their genotype at the kdr locus was determined using RFLP-PCR and HOLA protocols. A pronounced increase in resistance levels to all insecticides except CM had occurred across the test period, and it is readily apparent that resistance increased during spray periods. Concomitantly, we detected an increase in the frequency of the L1014F kdr mutation, especially in the M form in areas of industrial and biological cotton cultivation. We further report for the first time the occurrence of the L1014S kdr mutation we found floating at a low frequency in both the M and S forms of *An. gambiae* as well as in *An. arabiensis*. Analyses showed that the frequency of the L1014F kdr mutation is not statistically different in mosquitoes that died or survived to insecticide exposure, suggesting that the kdr mechanism might act together with other resistance mechanism(s) yet to be identified. Areas of extensive industrial and biological cotton cultivation are sustaining selection pressure for insecticide resistance in mosquito vector populations, prompting for collaboration between pest management in areas of cotton growing and vector control programmes to better face the challenge of increasing insecticide resistance in malaria mosquitoes.

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DYNAMICS OF INSECTICIDE RESISTANCE IN MALARIA VECTORS IN BENIN: FIRST EVIDENCE OF THE L1014S KDR MUTATION IN *ANOPHELES GAMBIAE* FROM WEST AFRICA

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Insecticide resistance monitoring is essential to help national programs to implement more effective and sustainable malaria control strategies in endemic countries. This study reported the spatial and seasonal variations of insecticide resistance in malaria vectors in Benin, West Africa. *Anopheles gambiae* s.l populations were collected from October 2008 to June 2010 in four sites selected on the basis of different use of insecticides and environment. WHO susceptibility tests were carried out to detect resistance to DDT, fenitrothion, bendiocarb, permethrin and deltamethrin. The synergist piperonyl butoxide was used to assess the role of non-target site mechanisms in pyrethroid resistance. *Anopheles gambiae* mosquitoes were identified to species and to molecular M and S forms using PCR techniques. Molecular and biochemical assays were carried out to determine kdr and Ace.1R allelic frequencies and activity of the detoxification enzymes.Throughout the surveys very high levels of mortality to bendiocarb and fenitrothion were observed in, *An. gambiae* s.l populations. However, high frequencies of resistance to DDT and pyrethroids were seen in both M and S form of *An. gambiae* s.s and *An. arabiensis*. PBO increased the toxicity of permethrin and restored almost full susceptibility to deltamethrin. *An. gambiae* s.l. mosquitoes from Cotonou and Malanville showed higher oxidase activity compared to the Kisumu susceptible strain in 2009 whereas the esterase activity was higher in the mosquitoes from Bohicon in both 2008 and 2009. A high frequency of L1014F kdr allele was initially showed in *An. gambiae* from Cotonou and Tori-Bossito whereas it increased in mosquitoes from Bohicon and Malanville during the second year. For the first time the L1014S kdr mutation was found in *An. gambiae* M form and in *An. arabiensis*. The ace.1R mutation was almost absent in *An. gambiae* s.l. Pyrethroid and DDT resistance is widespread in malaria vector in Benin and both metabolic and target site resistance are implicated. Resistance was not correlated with a change of malaria species and/or molecular forms. The L1014S kdr allele was first identified in wild population of *An. gambiae* s.s and *An. arabiensis* hence confirming the expansion of pyrethroid resistance alleles in Africa.

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INSECTICIDE RESISTANCE MANAGEMENT: THE KEY TO CONTINUED SUCCESS OF MALARIA VECTOR CONTROL IN ZAMBIA

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In the absence of a vaccine, insecticide-based vector control has been harnessed for prevention of malaria transmission in endemic countries. In Zambia indoor residual spraying (IRS) with DDT (2g/m^2) and pyrethroids (25mg/m^2) and insecticide treated nets (ITNs) are implemented as frontline interventions. However, their continued efficacy for successful and sustainable malaria vector control is threatened by emergence of resistance in *Anopheles* species in Africa. Studies to evaluate the spatiotemporal resistance profiles in malaria vectors were conducted in spatially segregated localities. A total of 4,581 F1 generation *An. gambiae* s,l (2,745) and *An. funestus* (1,836) were assayed for susceptibility using WHO standard discriminating dosages. Both Leu-Phe (west) and Leu-Ser (east) knock down resistance (kdr) mutations assays were investigated. By 2004, no resistance had been detected in either *An. gambiae* s.l. or *An. funestus* in Zambia. Between 2009 and 2011 significant levels of resistance to pyrethroids (0.05% deltamethrin, 0.05% lambda-cyhalothrin and 0.75% permethrin) and DDT (4%) were detected in both species ($p < 0.001$). High levels of Leu-Phe (west) kdr mutation and monooxygenases (P450) have been detected in *An. gambiae* s.s and *An. funestus* respectively. Marked levels of resistance were detected in IRS than in ITNs areas. No resistance was detected to the carbamate (0.01% bendiocarb) or the organophosphate (5% malathion) in either species. This implies that resistance selection is due to scaled up IRS and ITNs and could potentially undermine malaria control. This has resulted in a change of IRS policy from pyrethroids and DDT to carbamates. To preserve the limited arsenal of insecticides, good stewardship through a rational insecticide resistance management strategy is critical. Thus a strong partnership has been set up and data on potential underlying mechanisms of insecticide resistance, factors contributing to its emergence and distribution is being collated. This will ensure evidence-based choice of insecticides and their prolonged efficacy in Zambia.

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CHIKUNGUNYA INFECTION AMONG HOSPITALIZED FEBRILE PATIENTS IN NORTHERN TANZANIA

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Little is known about Chikungunya virus (CHIKV) as a cause of undifferentiated febrile illness in non-epidemic settings in sub-Saharan Africa. To investigate the prevalence of CHIKV infection, acute serum was collected from consecutive febrile inpatients at two hospitals in northern Tanzania from September 2007 to August 2008. Confirmed acute CHIKV infection was defined as a positive PCR result for CHIKV RNA. Among 870 participants, PCR testing was performed on 700 (80.5%). Of these, 55 (7.9%) had confirmed acute CHIKV infection. CHIKV infection was more common during dry months (OR 3.2, p=0.001) and cold months (OR 3.9, p<0.001), and was more common among infants and children than adults and adolescents (OR 1.9, p=0.026). Clinical signs and symptoms, hematologic results, and radiographic features were largely unhelpful in distinguishing CHIKV-infected patients from other febrile inpatients, with the exception of hepatomegaly (OR 2.3, p=0.043) and an absence of vomiting (OR 0.49, p=0.043). We report the first case series of patients with HIV and CHIKV co-infection. Among HIV infected patients, CHIKV infection was strongly associated with lymphopenia (OR 5.6, p=0.017) and severe immunosuppression (OR 10.5, p=0.007). The most common clinical diagnosis among CHIKV-infected participants was malaria in 23 (41.8%); no participant received a clinical diagnosis of CHIKV infection. Five CHIKV-infected patients died, one of whom likely had CHIKV meningoencephalitis. In conclusion, CHIKV infection is an important but unrecognized cause of febrile illness in northern Tanzania, even in the absence of a recognized outbreak. The preponderance of cases among pediatric participants observed is in marked contrast to the age distribution observed in outbreaks in non-immune populations outside of Africa. CHIKV infection was commonly misdiagnosed as malaria, however

CHIKV infection was more than twice as common as malaria in this study. Further research is needed to fully understand the epidemiology of this and other arboviruses in sub-Saharan Africa in non-epidemic settings.

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LABORATORY CONFIRMED PERINATAL TRANSMISSION OF DENGUE VIRUS IN PUERTO RICO

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Dengue is a mosquito-borne, acute febrile illness (AFI) with a 7-10 day period of viremia. During this time dengue virus (DENV) transmission can be blood-borne by receipt of blood products or donor organs or tissue and transmission from mother to fetus *in utero* or to infants at parturition (perinatal transmission). The determinants of perinatal transmission and the rate at which transmission occurs are unknown. Perinatal transmission may be under recognized in dengue endemic areas, including Puerto Rico. We present a laboratory confirmed, perinatal case of dengue from Puerto Rico. In 2010, a pregnant woman presented to a hospital with one day history of headache and fever. She went into labor while being evaluated and gave birth to a healthy male infant at 38 4/7 weeks gestation by vaginal delivery. Because the woman had been Group B Streptococcus (GBS) positive at 34 weeks, she and her newborn were evaluated and treated empirically for GBS infection. Although all cultures for GBS were negative, a diagnosis of dengue was entertained because the women had had 4 days of fever and thrombocytopenia and Puerto Rico was in the midst of a large dengue epidemic. The diagnosis of dengue in the mother was confirmed by RT-PCR for DENV-1 one week after delivery. The same day, the newborn, who had been well and about to be discharged from the hospital after completing empiric treatment for GBS, became hypoactive and thrombocytopenic. Serum was sent to CDC for DENV testing and RT-PCR was positive for DENV-1. Later the infant developed ascites, DIC and anemia, requiring fresh frozen plasma and packed red blood cell transfusions. During hospitalization, the infant acquired a nosocomial urinary tract infection. This case highlights that dengue should be included in the differential diagnosis for pregnant women with AFI living in dengue endemic areas. This case also highlights that during the neonatal period; signs other than fever may indicate DENV infection. Recognition of dengue in the mother is important to provide optimum management for both mother and infant.

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CLINICAL FEATURES THAT DIFFERENTIATE DENGUE FROM OTHER FEBRILE ILLNESSES AMONG CASES PRESENTING TO AN ACUTE CARE FACILITY IN A CARIBBEAN ENDEMIC AREA, JUNE 2009 - DECEMBER 2010

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Dengue, a mosquito-borne acute febrile illness (AFI), is endemic and reportable by law in Puerto Rico. Clinical diagnosis of the disease can be challenging because of its non-specific presentation and similarities with other AFIs. We examined clinical features of laboratory-positive (LP) and laboratory-negative (LN) dengue cases reported to a hospital-based enhanced dengue surveillance system (EDSS) from June 2009 to December 2010. AFIs that met World Health Organization criteria for dengue or severe dengue were reported to the EDSS via submission of a report form and serum sample. LP dengue cases had either detectable dengue virus (DENV) by RT-PCR or anti-DENV IgM by an enzyme-linked immunosorbent assay (MAC ELISA). LN cases had no evidence of DENV by RT-PCR and no detectable anti-DENV IgM. Cases with no DENV detected by RT-PCR and no convalescent specimen submitted for MAC ELISA were laboratory-indeterminate. Clinical and laboratory features that distinguish between LP and LN cases were evaluated. During the study period, 1634 suspected dengue cases were reported; 810 (50%) were LP, 327 (20%) were LN, and 497 (30%) were laboratory-indeterminate. LP cases were more likely than LN cases to have headache (82% vs.71%, p <0.0001), retro-orbital pain (63% vs. 48%, p <0.0001), body aches (79% vs. 66%, p <0.0001), joint pain (66% vs. 49%, p <0.0001), rash (38% vs. 28%, p =0.0015), petechiae (30% vs. 19%, p=.0005), hemorrhagic manifestation (41% vs. 33%, p=0.0224), thrombocytopenia (76% vs. 70%, p=0.0334), and leucopenia (66% vs. 57%, p=0.0044). In contrast, upper respiratory tract symptoms were more likely to be reported among LN cases than LP cases (63.5% vs. 36.5%, p< 0.0001). We plan to conduct a sensitivity analysis and generate receiver operator characteristic curves to determine the combination of clinical and laboratory features that best predict LP dengue cases among adults and children presenting with AFI by day of presentation. Findings will help to improve clinical detection of LP cases and guide clinical management.

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ETIOLOGY OF FEBRILE ILLNESSES IN NEPAL

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Acute febrile illness is a common reason for seeking medical care in Nepal. However, with a general unavailability of diagnostic tests, cases are frequently treated empirically with the underlying illness remaining undiagnosed. The determination of accurate year-round epidemiologic data for febrile patients and information regarding predominant symptoms for different diseases will assist clinicians in their diagnoses and subsequent therapeutic interventions even when laboratory resources are lacking. To this end, the Armed Forces Research Institute of Medical Sciences (AFRIMS) and the Walter Reed/AFRIMS Research Unit Nepal (WARUN) initiated a febrile illness etiology study at 4 hospitals in 3 cities in Nepal. Study methods included taking a standardized medical history and definitive diagnostic testing of acute and convalescent samples as appropriate for typhoid and paratyphoid fever, Japanese encephalitis (JE), dengue fever, chikungunya, West Nile virus, malaria, leptospirosis, rickettsiosis, influenza, brucellosis, hepatitis A, B, C and E, and bartonellosis. From May 2009 to December 2010, we enrolled 2,046 patients presenting with an undifferentiated febrile illness with no known etiology. The average age was 26 (range 2-96 years). Testing results to date have demonstrated 69 infections with *Salmonella typhi*, 73 *Salmonella paratyphi* A, 13 malaria, 204 leptospirosis, 15 hepatitis A, 1 hepatitis B, 1 hepatitis C, 62 brucellosis, 1 chikungunya, 47 primary dengue, 47 secondary dengue, 12 JE, 7 murine typhus, 1 Thai tick typhus, 52 scrub typhus, 130 influenza A/H1N1, 6 A/H3, 167 influenza B, 2 *Bartonella henselae* and 5 *B. quintana*. These are the first known reports of both Chikungunya and Bartonella human infections in Nepal. In addition, although only documented in Nepal since 2004, dengue infections are now being seen in the cities of Kathmandu and Pokhara located at higher altitudes further from the Indian border than initial cases. Characterization of the infections and correlation with clinical symptoms is continuing in order to provide information to Nepalese healthcare providers to assist with empirical diagnosis and treatment and priorities for future diagnostic needs.

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THE ETIOLOGY OF ACUTE FEBRILE ILLNESS IN PATIENTS PRESENTING TO GARISSA PROVINCIAL HOSPITAL IN NORTHEASTERN PROVINCE, KENYA

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Acute febrile illness (AFI) is a common clinical syndrome among patients in North Eastern Kenya. The non-specificity of the signs and symptoms of these illnesses leads clinicians to assign a presumptive malaria diagnosis in the absence of supportive laboratory testing and alternative diagnostic considerations. We report on other etiologies that should form a basis for differential diagnosis of fever in this region. The study population came from a cross sectional observational study of 304 patients presenting with non-malarial fever of ≥ 38°C at the Garissa Provincial Hospital in North Eastern Province, Kenya for 12 months during 2009-2010. Malaria was excluded by examining thick and thin Giemsa stained blood smears. Total nucleic acid (RNA and DNA) were extracted and assessed for Salmonella, malaria, Brucella, Leptospira and Rickettsia by a PCR strategy (RT-qPCR) that amplifies total nucleic acid following reverse transcription. Of the 304 AFI cases, 107 (35%) had identifiable pathogens: *Brucella* spp were found in 45 (14.8%), *Salmonella* spp in 39 (12.8%), and *Rickettsia* spp in 20 (6.57%). No leptospira infection was detected. Co-infections were observed in 12 (3.9%) patients while triple infections were observed in 2 (0.7%) patients. Additionally, despite the samples being negative for malaria by microscopy, 18 patients (5.9%) tested malaria positive by real-time RT-qPCR. Other diseases such as brucellosis, salmonellosis and rickettsiosis should be considered in cases of AFI. Testing for more etiologies such as Rift Valley fever, Coxiella spp, and arboviruses are important as less than half of the AFI cases were laboratory confirmed.

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HANDHELD POINT-OF-CARE CEREBROSPINAL FLUID LACTATE TESTING PREDICTS BACTERIAL MENINGITIS IN UGANDA

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Bacterial meningitis (BM) contributes to a high burden of morbidity and mortality in resource limited settings. Diagnosis may be delayed in part due to lack of human and material resources. Therefore, we validated a handheld point of care lactate (POCL) monitor's ability to measure lactate in cerebrospinal fluid (CSF) and diagnose BM in Uganda. Using a handheld POCL monitor we prospectively evaluated (in duplicate) 98 consecutive CSF samples submitted for standard laboratory lactate (SLL) testing at the University of Virginia. After the validation step, 145 patients with suspected BM were evaluated in Mbarara, Uganda. Probable BM was defined as a CSF white blood cell count of 100 cells of which 50% were neutrophils in the absence of an alternative diagnosis. Proven BM was defined by positive CSF Gram's stain or isolation of bacteria from CSF or blood. The ability of CSF POCL to diagnose BM was assessed by receiver operating characteristic (ROC) curves. Statisitcal significance was set at $p < 0.05$. There was a strong linear correspondence between CSF POCL and SLL test results ($R^2 = 0.86$; $p < 0.001$). There was a slightly higher CSF SLL (mean = 2.89 mmol/L, SD ± 1.91) compared to POCL average concentration (mean = 2.33 mmol/L, SD ± 1.92; mean difference, 0.56 mmol/L; $p < 0.001$). A CSF POCL of ≥ 7.7 mmol/L provided 94% sensitivity and 90% specificity for BM [AUROC = 0.95, 95% CI (0.9-1.0), $p < 0.001$]. The same value provided 100% sensitivity and 88% specificity for proven BM [AUROC = 0.96, 95% CI (0.91-1.0), $p < 0.001$]. CSF POCL was not helpful in the diagnosis of cryptococcal meningitis [AUROC = 0.48, 95% CI (0.39-0.58), $p = 0.73$]. As CSF POCL values increased, the likelihood of a tuberculous meningitis diagnosis decreased [AUROC = 0.338, 95% CI (0.24-0.44), $p = 0.005$]. In conclusion, a CSF POCL concentration of ≥ 7.7 mmol/L differentiated BM from other causes of meningitis with high sensitivity and specificity in a quick and easily obtainable manner. Use of CSF POCL testing may improve management of patients with suspected meningitis where laboratory infrastructure is limited.

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RISK FACTORS FOR DEATH AND SEVERE SEQUELAE IN MALAWIAN CHILDREN WITH BACTERIAL MENINGITIS, 1997-2010

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Acute bacterial meningitis causes significant death and disability in children worldwide and HIV is an established risk factor for acquiring meningitis and suffering negative outcomes. We investigated risk factors associated with death and severe sequelae in Malawian children with bacterial meningitis. A retrospective database review of three previous studies of acute bacterial meningitis was conducted on 1,784 children less than 15 years of age who attended Queen Elizabeth Central Hospital in Blantyre, Malawi during 1997--2010. Multivariate logistic regression was used to estimate the effects of HIV seropositivity, impaired consciousness, and causative organism on death and severe sequelae after adjusting for additional risk factors, including nutritional status, age, anemia, and *Plasmodium falciparum* infection. Impaired consciousness or coma at the time of admission was strongly associated with death [Coma: OR = 14.4, 95%CI (9.42, 22.1)] and severe sequelae [Coma: OR = 3.27, 95%CI (2.02, 5.29)] in multivariate logistic regression models. HIV seropositivity was significantly associated with increased odds of death [OR = 1.65, 95%CI (1.20, 2.26)] but no association was observed for developing severe sequelae [OR = 0.88, 95%CI (0.56, 1.38)]. After adjustment, infection with Salmonella spp was associated with increased odds of death [OR = 2.11, 95%CI (1.06, 4.08)] and pneumococcal meningitis was associated with increased odds of severe sequelae [OR = 1.84, 95%CI (1.03, 3.29)]. Resistance to commonly used antibiotics was not associated with increased risk of death after adjustment for causative organism and HIV serostatus, but the proportion of Streptococcus pneumoniae and Hemophilus influenzae type b strains resistant to co-trimoxazole increased over the period of study. Based on these findings, we conclude that impaired consciousness and HIV infection are major risk factors for death from ABM in Malawian children. Use of the pneumococcal conjugate vaccine could greatly reduce the burden of ABM in Malawi.

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BIOMPHALARIA GLABRATA PLASMA PROTEINS WITH BINDING AFFINITY TO A MEMBRANE-ENRICHED FRACTION OF SCHISTOSOME PRIMARY SPOROCYSTS: A PROTEOMIC ANALYSIS

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Upon entry of infective *Schistosoma mansoni* miracidia into its snail intermediate host *Biomphalaria glabrata*, these larval stages are immediately exposed to hemolymph, comprised of both soluble plasma and circulating hemocytes. Aside from providing an environment conducive to transformation to the primary sporocyst, hemolymph also plays an important role in ultimately determining the immune compatibility between snail host and establishing larval infection. Both

plasma and hemocyte components are involved, although the complex interaction between the parasite and these immune elements is still poorly understood. In order to understand more clearly this interaction at the molecular level, we have employed an enriched fraction of biotinylated *S. mansoni* sporocyst membrane proteins immobilized to avidin-conjugated beads as an affinity matrix, for the purpose of isolating sporocyst-binding proteins from the plasma of a susceptible (NMRI) and resistant (BS-90) strain of *B. glabrata*. Isolated samples were subjected to "in liquid" digestion and nanoLC-MS/MS analysis using the Agilent 1100 nanoflow system connected to a hybrid linear ion trap-orbitrap mass spectrometer equipped with a nanoelectrospray ion source. Raw MS/MS data was searched against the *B. glabrata* supercontig genome database (ver. 4.0.1) translated into 6-reading frames using an in-house Mascot search engine. Fibrinogen-related proteins (Freps) were the predominant protein group identified including Frep2 (2.13, 2.19, 2.22, 2.25, 2.29), Frep 3 (3, 3.2, 3.3, 3.3pre, 3-2pre), Frep7 (7, 7.1), Frep 12 (12.1, 12.1pre), and Frep 13 (13.1, 13.1pre). All putative Frep sequences were detected in both *B. glabrata* strains except Frep2, which was only recovered from NMRI snail plasma. Other putatively identified plasma proteins included dermatopontin2, a selectin and hemoglobin types1 and 2 in both snails, and Ca-binding protein2 in NMRI plasma. This approach not only provides putative identification of host plasma proteins interacting with the sporocyst membrane, but also will contribute to ongoing efforts to annotate the current *B. glabrata* genomic sequence database.

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HIGH-THROUGHPUT RNA-SEQ OF *SCHISTOSOMA MANSONI* TRANSCRIPTOME

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Schistosoma mansoni is one of the agents of schistosomiasis, a chronic and debilitating disease. In the past seven years, two sequencing projects have contributed a considerable amount of molecular information on the parasite, covering a significant portion of both the transcriptome and the genome, as published previously. The most recent estimate of the number of genes is 13,207. However, the fragmented nature of the data is still apparent, as 2,836 genes predicted in the genome have no evidence of transcription in the available EST databases, while approximately 7,000 EST contigs in the public databases do not map to the genome sequence and/or to the predicted genes. Recent advances in next-generation sequencing technology promise to accelerate the acquisition of sequences and diminish the cost of sequencing of large and complex genomes as well as of transcriptomes. In the present work, we used Roche 454 pyrosequencing to explore the *S. mansoni* adult male transcriptome (RNA-seq). A total of over 1.6 million high-quality ESTs were obtained from adult males with average length = 232 nt (40-1,433 nt) from the 3'-end of messages, resulting in a 26 % higher coverage of genome bases than that of public ESTs available at NCBI. With a 15 X-deep coverage of transcribed genomic regions, our data were able to (i) confirm for the first time 990 predictions without previous evidence of transcription; (ii) correct gene predictions; (iii) identify 11 new Micro-exon Genes (MEGs); (iv) discover 989 and 1196 RNA-seq contigs that map to intergenic and intronic genomic regions, respectively, where no gene had been predicted before. These contigs could represent new protein-coding genes or non-coding RNAs (ncRNAs). High-throughput RNA-seq of *S. mansoni* adult males helped uncover the parasite transcriptome complexity.

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USING COMPARATIVE FUNCTIONAL GENOMICS TO IDENTIFY NOVEL THERAPEUTIC TARGETS IN *SCHISTOSOMA MANSONI*

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Schistosomiasis is a tropical disease caused by flatworm parasites, *Schistosoma*, that affects hundreds of millions of people in the developing world. Although only a single drug (praziquantel) is available to treat this disease, the complicated life cycle of this parasite, that involves both mollusc and vertebrate hosts, impedes efforts to uncover and validate novel therapeutic targets. Thus, we are exploring the utility of the planarian *Schmidtea mediterranea*, a free-living relative of *Schistosoma*, to serve as an experimentally tractable model to identify and characterize new anthelmintic targets. We previously reported that a peptide hormone, NPY-8, is required for the maintenance of reproductive organs in the planarian and showed that a close relative of this hormone is present in the genome of *S. mansoni*. Since the major cause of the pathology associated with schistosome infection is a result of their prodigious reproductive output (100-3000 eggs/day), we are exploring whether this class of hormones functions similarly in these parasites. Additionally, we used comparative genomics to identify genes highly conserved between the planarian and the schistosome that are not found in the genomes of mammals. This analysis uncovered hundreds of genes, many of which encode "drugable" targets including G protein-coupled receptors, ion channels, and enzymes. We are using high-throughput in situ hybridization and RNA interference to characterize these genes in the planarian; follow-up analyses for a subset of genes will be performed in the parasites. Together, our data highlight new opportunities for translating knowledge gained from studying planarians to understand and control parasitic disease.

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GENETIC KNOCKDOWN OR PHARMACOLOGICAL INHIBITION OF *SCHISTOSOMA MANSONI* MULTIDRUG RESISTANCE TRANSPORTERS DISRUPTS PARASITE EGG PRODUCTION

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P-glycoprotein (Pgp) and multidrug resistance-associated protein 1 (MRP1) are members of the ATP-binding cassette (ABC) superfamily of proteins involved in transport of toxins and xenobiotics from cells. These transporters are associated with development of multidrug resistance (MDR) in mammals, and have been implicated in resistance to antiparasitic drugs, including anthelmintics. They likely also play key physiological roles in the parasite's excretion of wastes and metabolites, and provide attractive candidate targets for novel antischistosomal agents. We have previously shown that expression of *Schistosoma mansoni* Pgp (SMDR2) and MRP1 (SmMRP1) is altered in worms exposed to praziquantel (PZQ), the current drug of choice against schistosomiasis, and that higher expression is associated with reduced susceptibility to PZQ. We have also shown that PZQ inhibits SMDR2, and is also a likely substrate of SMDR2. We are currently using molecular genetic and pharmacological approaches to define the physiological roles played by these transporters and to dissect the mechanisms by which they interact with PZQ and may modulate responsiveness to the drug. RNA knockdown of SMDR2 or SmMRP1 in adult *S. mansoni* results in disruption of egg production by worms in culture. Exposure of adult worms to a variety of Pgp and MRP1 inhibitors, including tariquidar, a highly selective, third generation Pgp inhibitor, also produces significant disruption of egg production, as does exposure to MK 571, a MRP1 inhibitor. Treatment of *S. mansoni*-infected mice with MDR inhibitors results in reduced liver egg burden. We are currently examining the mechanism underlying this disruption of egg

production. Our findings indicate that these transporters may be excellent candidate targets for new anthelmintic strategies, either on their own or as an adjunct to currently available therapeutics.

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EFFECT OF HUMAN TGF-SS ON THE GENE EXPRESSION PROFILE OF *SCHISTOSOMA MANSONI* ADULT WORMS

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Schistosoma mansoni is responsible for schistosomiasis, a parasitic disease that is a major cause of morbidity worldwide. Molecular mechanisms of host-parasite interaction are complex and involve a crosstalk between host signals and parasite receptors. Transforming Growth Factor Beta (TGF-ß) is a cytokine that regulates many process central to life of metazoans such as growth and differentiation, developmental patterning, tissue repair and cell death. TGF-β signaling pathway has been shown to play an important role in *S. mansoni* development and embryogenesis. In particular human (h) TGF-β has been shown to bind to a *S. mansoni* receptor, transduce a signal that regulates the expression of a schistosome target gene. In spite of evidence of a TGF-ß effect on schistosome biology, limited information is available on which genes are affected at the transcriptional level. In this work we present the effect of human TGF-ß on the gene expression profile of adult worms by identifying 2167 parasite genes whose expression levels are affected by *in vitro* treatment of adult worms with hTGF-β. Among these differentially expressed genes, we highlight genes related to development, cell cycle and embryogenesis that could be players of hTGF-β effects on the parasite. We confirm by qPCR the expression changes detected with microarrays for 6 out of 8 selected genes. We also highlight a set of non-coding RNAs transcribed from the same loci of protein-coding genes that are differentially expressed upon hTGF-β treatment. These datasets offer potential targets to be explored in order to understand the molecular mechanisms behind the role of hTGF-β effects on parasite biology.

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CHARACTERIZATION OF NOVEL GLUTAMATE RECEPTORS IN *SCHISTOSOMA MANSONI*

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Cys-loop ligand-gated ion channels (LGIC) are instrumental for nervous system modulation, both in vertebrates and invertebrates. Very little is known about the LGICs in the parasitic platyhelminth *Schistosoma mansoni*, even though several LGIC gene sequences are predicted from its genome. Our work focuses on 3 of these LGIC, which we have previously cloned and identified as glutamate-gated ion channel (GluCl) subunits. Further characterization of these GluCl subunits by two-electrode voltage clamp (TEVC) in X. laevis oocytes reveals that the pharmacological and biophysical properties of these GluCls is distinct from their counterparts in other invertebrates, particularly with regard to their sensitivity to agonists and modulators. In addition, confocal laser microscopy analyses show that these SmGluCl subunits are distributed throughout the central and peripheral nervous system of the worm. These new findings have important implications for the fundamental comprehension of the key roles played by neuronal modulation in the parasite life style. More importantly, these SmGluCl channels constitute a very attractive novel drug target and could be used for screening and development of new anthelmintics.

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A NEW GOLD STANDARD FOR DIAGNOSIS OF *SCHISTOSOMA HAEMATOBIUM*

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Inconsistent sensitivity and specificity among current diagnostic procedures has made it difficult to set a gold standard for the definitive diagnosis of *Schistosoma haematobium* (Sh) in people with low level or chronic infections. These people are often missed because they often pass few eggs in the urine. Our study explored an alternative diagnostic method based on the presence of Sh-specific *Dra*1, 121 bp repeat DNA fragments in human urine and introduced a novel method of collecting and filtering urine specimens using Whatman No. 3 filter paper and drying them in the field for easy transport to the laboratory and subsequent examination. Research was performed in two sets: 1) Three diagnostic tests were used to examine 89 urine specimens from school children in Kollo District, Niger: dipsticks to detect hematuria (DM) in urine, microscopic detection of Sh eggs (ME) on the paper surface, and PCR for detection of Sh *Dra*1 extracted from the paper (PCR). In all 52 (58.4%) showed hematuria, 44(49.4%) showed eggs and 51(57.3%) showed Sh-specific DNA. 2) Latent Class (LC) modeling was used to compare the performance of the three tests in 401 filtered urine specimens from unselected adults (aged 20 - 59 years) from six endemic villages in Ogun State, Nigeria: PCR was superior with specificity of 1.000 and sensitivity of 1.000, ME had specificity of 1.000 and sensitivity of 0.701, while DM had specificity of 0.857 and sensitivity of 0.955. DM also showed a difference between males and females. LC modeling enabled the evaluation of specificity and sensitivity of a test when the actual prevalence of the pathogen is unknown. In Kollo and Ogun several persons had DNA detected in urine in the absence of detectable eggs; PCR product was not dependant on the egg count; the schistosome-specific DNA was undetectable in 61 previously positive people treated and re-examined 14 days later. Filter paper samples remained potent for at least four months at room temperature. This makes field collection of urine convenient and simple.

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SAFETY AND EFFICACY OF PRIMAQUINE WHEN COMBINED WITH QUININE OR DIHYDRO-ARTEMISININ PLUS PIPERAQUINE FOR RADICAL CURE OF *VIVAX* MALARIA IN INDONESIA

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The efficacy of primaquine against relapse has not been reliably assessed since the drug was developed in clinical trials in American prisoners during the 1950s. Two linked problems compound the difficulty of assessment of therapeutic efficacy of primaquine in endemic zones: 1) the long duration between primary infection and risk of relapse; and 2) the inability to distinguish relapse from reinfection among recurrent parasitemias in subjects under long-term follow-up. We screened Indonesian soldiers returning to their base in malaria-free East Java after serving 11 months in heavily malarious northeastern Papua, Indonesia. Among 143 found positive for *Plasmodium vivax*, 116 were randomized to three treatment groups: 1) artesunate alone; 2) quinine + primaquine (0.5mg/kg/dayX14d); or 3) Dihydro-artemisinin/piperaquine (DHA-PP) + primaquine (0.5mg/kg/dX14d). Treatment was directly observed, and subjects will be followed until first recurrence of parasitemia, or for 12 months. At submission of

this abstract, subjects had been under observation for 8 to 147 days. Relapses had occurred among 30 of 41 subjects given artesunate alone, and the median day of relapse was day 21 post-patency (range 17 to 70 days). This provides a measure of the natural rate of relapse and permits calculation of primaquine efficacy against it. Relapses occurred among 5 of the 39 subjects given quinine + primaquine, and the median day of relapse was day 70 post-patency (range 35 to 104 days). Two relapses had occurred among the 36 subjects randomized to DHA-PP +primaquine, on day 82 and day 126 post-patency. The efficacy against relapse of DHA-PP +primaquine appears superior to quinine + primaquine. This study may demonstrate good safety and efficacy of an adult dose of 30mg primaquine daily for 14 days against relapse when administered with DHA-PP against the acute attack. The observed difference in efficacy of the same dose of primaquine against relapse when given with quinine or DHA-PP emphasizes the apparent impact of that a blood schizontocide may have on primaquine activity against hypnozoites.

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WHAT IS THE APPROPRIATE SECOND LINE REGIMEN IN THE ERA OF ARTEMISININ COMBINATION THERAPY: EFFICACY OF QUININE, ARTEMETHER-LUMEFANTRINE AND DIHYDROARTEMISININ-PIPERAQUINE FOR RECURRENT UNCOMPLICATED MALARIA IN UGANDAN CHILDREN

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Falciparum malaria therapy poses unique challenges in sub-Saharan Africa, where recurrent infections are common, especially in children. Though in several countries quinine is the recommended treatment for these patients, it is unclear whether this is the best approach. The study was a nested, randomized, open label, three-arm clinical trial of rescue therapy among patients who developed recurrent malaria within 28 days following treatment of the primary episode with an artemisinin-based combination treatment (ACT) in a related main study. Consecutive patients aged 6 to 59 months with recurrent uncomplicated malaria were randomised to receive either quinine or one ACT, i.e. artemether-lumefantrine (AL) or dihydroartemisinin-piperaquine (DHAPQP), and actively followed up for the next 28 days. Among 220 patients enrolled, 217 (98.6%) were assigned an efficacy outcome and 218 (99.1%) were assessed for safety. Risk of recurrent infection was significantly higher for quinine (70% [74/110], HR 3.9, 95%CI 2.4-6.7, p<0.0001) and AL (60% [21/35] HR 3.3, 95%CI 1.8-6.3, p<0.0002) as compared to DHAPQP (25% [18/72]). When adjusted by genotyping, risk of treatment failure was lower in the DHAPQP group (1% [1/72]) compared to quinine (7% [8/110]) and AL (5 % [2/35]) group, though not statistically significant. No serious adverse events were reported. A recurrent infection following an ACT treatment can be successfully treated with an alternative ACT instead, than with quinine, the current recommended second line regimen in Uganda and in 29 other African countries. An ACT rather than quinine should be used as second line treatment.

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ASSESSMENT OF THE EFFICACY, TOLERABILITY AND EASE OF ADMINISTRATION OF DIHYDROARTHEMISININ PLUS PIPERAQUINE AND ARTESUNATE PLUS SULFAMETHOXYPYRAZINE PLUS PYRIMETHAMINE COMPARED WITH SULPHADOXINE-PYRIMETHAMINE FOR PREVENTING MALARIA IN GHANAIAN CHILDREN

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Seasonal administration of intermittent preventive treatment for malaria given to children under five years old (IPTc) involves administration of a pre-defined number of treatment courses of antimalarial drugs at specified time intervals during the high transmission season. Recent reports indicate that IPTc is safe and can reduce the burden of malaria in West Africa. Using different drug combinations for IPTc will minimize the development of resistance to first line drugs. Factors such as side effects, ease of administration, duration of the treatment, become important, when selecting the appropriate treatment for IPTc. A combination of antimalarial drugs with efficacy lasting over 42 days would be of great importance for IPTc. We investigated the efficacy, longevity, tolerability and ease of administration of dihydroarthemicinin plus piperaquine (DHA+PQ), artesunate plus sulphamethoxypyrizine plus pyrimethamine (Co-Arinate FDC®) 12 hourly over 24 hours, Co-Arinate FDC® daily for three days and compared with Sulphadoxine-pyrimethamine (SP) in children aged 6-59 months with asymptomatic malaria in Ghana. An open labelled, active, controlled, randomized Phase III trial with four arms was used. A total of 590 children from 28 villages were randomly assigned to the four arms. One arm (148) received DHA+PQ daily for three days, the second arm (143) received Co-Arinate FDC®) daily for three days, the third arm (149) received Co-Arinate FDC ® 12 hourly over 24 hours and SP the comparator arm (150) received a single dose. The children were followed up to 63 days. finger prick blood was collected for blood film and filter paper on days 0, 3, 7, 14, 28, 42 and 63 for parasite identification. Safety was assessed by visiting subjects at home from day 0 to 7, haemoglobin concentration was measured on days 0, 14, 28, 42 and 63 and venous blood for liver and renal function tests collected on days 0 and 14. Ease of drug administration was assessed by interviewing parents/guardians. Treatment failure (PCR-uncorrected) by day 42 was SP 40%, Co-Arinate daily 26.6%, Co-Arinate 12hourly 34.9% and DHA+PQ was 16.2%. Vomiting was more common among children in the Co-Arinate 12hourly arm 18.1% compared to SP 6.7%, Co-Arinate daily 11.2% and DHA+PQ 8.1%. The intervention was found to be acceptable to the community. Our findings show that DHA+PQ and Co-Arinate daily are safe and efficacious for IPTc in Ghanaian children.

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STAGE SPECIFIC CLEARANCE OF ASEXUAL *PLASMODIUM FALCIPARUM* IN CHILDREN TREATED WITH ARTESUNATE-AMODIAQUINE (AA) AND ARTEMETHER-LUMEFANTHRINE (AL)

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Delay in parasite clearance time is a recognized indicator of emerging drug tolerance. Artemether-lumefantrine (AL) and Artesunate-amodiaquine (AA) are efficacious regimens that have been widely adopted in sub-Saharan Africa. We evaluated their clearance times on the asexual stages

of *Plasmodium falciparum* using Giemsa stained thick blood films. This is preliminary to evaluating stage specific delay in parasite clearance time that may be a more sensitive indicator of parasite tolerance to the antimalarial therapies. Children aged 7 months to 12 years were randomized to receive standard doses of AA and AL for three days. Peripheral blood smears were made hourly in the first 4 hours, 8h, 16h, 24h, and on days 2-7, 14, 21, 28, 35, and 42 for microscopic identification, quantification, and morphological staging of *Plasmodium falciparum*. The appearance and ratio of the parasite nuclear chromatin and cytoplasm were the characteristics used in the staging. Parasites were classified into R1 (very young rings, 0-6 hrs), R2 (young trophozoites, 6-30 hrs), and R3 (late trophozoites, >30 hrs). Schizonts were classified as immature (Si, <8 visible nuclear chromatins) and mature (Sm, > 8 nuclear chromatins). A total of 57 (28AA, 29AL) children were evaluated. Thirty four (59.6%) of the children had multiple stages of the parasite in their blood films at enrolment. The average number of stages per child at enrolment was 2. R1 was seen in Forty seven children (82.5%), while 49.1% and 38.6% had R2 and R3 respectively. Pure R3 infection was the least common (5.3%). Low density schizontinaemia (6 - 120 Schizonts/µL) was present in 36.8% of the children before treatment (21.1%, 17.5%% immature and mature Schizonts respectively). Schizonts were more likely to be present in younger than older children [5.7 (± 2.5) vs. 8.1 (±2.7) years, p= 0.002]. Stage specific parasite clearance times (Log10 hours ± SD) for R1, R2, R3, Si, and Sm, were 1.5 ± 0.2, 0.9 ± 0.3, 0.6 ± 0.3, 0.6 ± 0.3, and 0.7 ± 0.3 for AA and 1.6 ± 0.2, 1.1 ± 0.3, 0.8 ± 0.4, 0.6 ± 0.5, and 0.7 ± 0.2 for AL respectively. All parasites were cleared by 72 hours of commencing therapy. The evaluated ACTs had more rapid actions on older stages of the parasite and Schizonts. In addition, the study revealed that low density schizontinaemia is not uncommon in children with acute uncomplicated *falciparum* malaria.

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PHARMACOKINETIC PREDICTORS OF TREATMENT OUTCOME FOR DIHYDROARTEMISININ-PIPERAQUINE IN UGANDAN INFANTS WITH UNCOMPLICATED MALARIA

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Dihydroartemisinin-piperaquine (DP) is the most recently adopted 1st line artemisinin-combination therapy (ACT) option for the treatment of malaria. We evaluated the pharmacokinetics (PK) and pharmacodynamics (PD) of piperaquine (PQ) in 107 infants, aged 6 to 24 months, within the context of a longitudinal study in the high transmission area of Tororo, Uganda. Capillary plasma samples were collected prior to the 1st dose of DP, and at variable times up to day 28 after each treatment for *P. falciparum* malaria. Children were followed longitudinally, and underwent sampling for all episodes of malaria. 218 episodes of malaria (1314 samples) occurred over the 7 month study period. Median day 7 concentrations were 41.9 ng/ml (IQR, 30.2, 56.6), Univariate and multivariate analyses revealed that day 14, 21, and 28 levels were significantly associated with the risk of recurrent malaria on day 42. The risk of recurrent infection increased 85% per log10 increase in PQ level on day 14. Those individuals with a PQ level in the lowest quartile (<10.5 ng/ml) on day 28 had a 41% risk of failure, while those above that threshold had a 20% cumulative risk of failure at 42 days (p=0.01). Notably, out of 132 children who had a prior episode of malaria treated with DP, 119 had detectable PQ at the time of diagnosis of their next episode of malaria (constituting a period of up to 4 months), with concentrations ranging from 1.5 to 41.9 ng/mL. Population PK analysis was also performed. A three compartment PK model with first order absorption of drug and age-dependent apparent clearance best described PQ disposition in infants. Additional population PK/PD analyses will be presented. Our study provides the 1st data on the disposition of PQ in children < 2 yrs of age. PQ exposure on day 7 for infants is lower than day 7 levels previously reported in older children and adults. Moreover, PK exposure correlates strongly with clinical outcomes. In addition, PQ appears to remain detectable for extended periods, and was detectable in the majority of infants upon recurrent infection.

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ARTEMETHER-LUMEFANTRINE EFFICACY IN PREGNANCY: THE PROOF IS IN THE PLACENTA

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Data on efficacy of artemisinin based combination therapy to treat *Plasmodium falciparum* during pregnancy in sub-Saharan Africa is scarce. In an antenatal cohort of women in Mbarara, Uganda, a recent open label, randomized, non-inferiority trial demonstrated that artemether-lumefantrine (AL) is non-inferior to quinine with an improved side effect profile. To determine whether AL is associated with reduced pathology compared to quinine to treat uncomplicated malaria in pregnancy, malaria pigment deposition and inflammation were assessed by histology in this cohort. Pigment deposition in fibrin and placental inflammation were scored on blinded hematoxylin and eosin and Giemsa stained placental sections. Clinical data included treatment arm, parity, gestational age of first parasitemia, level of parasitemia, and day of reinfection or recrudescence. The prevalence and amount of pigment proportionately decreased with time after treatment and this decline was greater in the AL arm. AL (n=61) was associated with decreased pigment compared to quinine (n=62), correcting for parity, time of infection, reinfection/recrudescence, and level of parasitemia (p=0.003). The prevalence of intervillous inflammation in this cohort was low (6%), reflecting the efficacy of antenatal care with early detection and prompt treatment of malaria. In conclusion, AL is associated with less malarial pigment deposition compared to quinine for treatment of uncomplicated malaria in pregnancy, suggesting that AL is more effective. This may reflect the increased rates of parasitologic clearance and activity of AL at early stages of the parasite life cycle. Histology may act as a surrogate outcome in drug efficacy trials during pregnancy with limited sample size, and would be a useful tool to evaluate malaria control policy and implementation in pregnancy.

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HIGHLY EFFECTIVE THERAPY FOR MALARIA IN PREGNANCY IMPROVES MATERNAL AND NEONATAL HEALTH OUTCOME

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Pregnant women are affected by the adverse outcomes of malaria but treatment options are limited. Dihydroartemisinin-piperaquine (DHP) is a safe and highly effective antimalarial in non-pregnant adults but limited information is available on its use in pregnancy. We report the safety profile of DHP exposures in pregnancy and the impact on pregnancy

outcomes following change in antimalarial treatment policy to artemisinin combination therapy. From April 2004 to June 2009, 6519 pregnant women were enrolled in a hospital based malaria surveillance study. All pregnant women were screened for malaria and treated. Eligible data for the safety analysis were available in 1217 pregnant women with acute antimalarials exposures on hospital admission (765 exposed to DHP) and 847 women with history of antimalarial exposures during the current pregnancy (395 with prior DHP exposures). Compared with prior quinine or chloroquine+/- sulfadoxine-pyrimethamine exposures, history of DHP treatment during the current pregnancy reduced the risk of recurrent malaria at delivery (OR=0.37 (95%CI 0.27-0.52), p<0.001), congenital malaria (OR=0.06, [95%CI, 0-0.46], p=0.001) and perinatal deaths (AOR=0.32 [95%CI, 0.12-0.85]; p=0.03) when used in the second and third trimester of pregnancy. There was no increased risk of congenital malformations and stillbirths in pregnant women exposed to DHP. The introduction of DHP was associated with a 53% fall in the overall proportion of maternal malaria at delivery and 94% reduction of congenital malaria incidence. In conclusion, DHP has an acceptable safety and toxicity profile for the treatment of malaria in the second and third trimester of pregnancy and reduces associated morbidity and mortality. Further prospective studies are required to define the role of DHP for the treatment and prevention of malaria in this high-risk group. Ensuring universal access to ACT in pregnancy through novel treatment and prevention strategies is likely to impact significantly on maternal child health.

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PHLEBOTOMINE BLOODMEALS IN A PERIURBAN LEISHMANIA-ENDEMIC AREA IN NORTHEASTERN BRAZIL

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Visceral leishmaniasis (VL) is caused by *Leishmania infantum chagasi* in northeastern Brazil. It is transmitted by *Lutzomyia* longipalpis and dogs are thought to be the major reservoirs. The study objective was to assess the role of *Leishmania*-infected humans in transmission by determining the source of sand fly bloodmeals. Study site was an *L. i. chagasi* - endemic periurban neighborhood of Natal, RN, Brazil. A subsection of 10% of this neighborhood (n=120) has had DTH and antibody testing; 38 (31.7%) were positive for one of these tests. From February to April 2011, sand flies were collected with CDC light traps in chicken enclosures, dog runs, and houses with prior but no active VL cases. Female sand flies were macerated and DNA extracted (Qiagen). *Lutzomyia longipalpis*-specific GAPDH, *Leishmania* minicircle kDNA, and species-specific cytochrome C to identify bloodmeal DNA were amplified by PCR and identified by agarose gel electrophoresis. A selection of PCR products were sequenced for confirmation. 244 sandflies were collected of which 93 were *L. longipalpis* by PCR. 19 of 139 (13.7%) sand flies were positive for *Leishmania* kDNA by PCR; 11 of 47 (23.4%) of *L. longipalpis* were kDNA-positive compared with 8 of 92 (8.7%) of non-*L. longipalpis.* Human blood alone was present in 40/233 (17.2%), both human and chicken blood in 11/233 (4.7%), chicken alone in 9/233 (3.9%), and human and dog in 5/233 (2.1%). *L. longipalpis* were nearly ten times more likely than non-*L. longipalpis* to have a human, dog, and/or chicken blood meal identified, 67.9% (57/84) vs. 7.4% (11/149), respectively. Of the 19 kDNA positive sand flies, seven had detectable human DNA. In this population, sand flies, particularly *L. longipalpis,* had fed on humans more than chickens despite the greater density of chickens compared with humans. Although kDNA positivity does not denote *Leishmania* infectivity, the presence of human blood in a greater percentage of kDNA-positive sand flies than chicken or dog blood suggests that asymptomatic human carriers may be important reservoirs of infection.

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THE INFLUENCE OF STREETS ON THE SPATIAL DISTRIBUTION OF THE CHAGAS DISEASES VECTOR, *TRIATOMA INFESTANS*, IN THE CITY OF AREQUIPA, PERU

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Urban transmission of Chagas disease is a documented problem in Southern Peru and elsewhere. While the dispersal of the vector, *Triatoma infestans,* has been well described in rural habitats, little is known about how the vector moves through the urban environment. Do city streets serve as a barrier to dispersal of *T. infestans*? And, if so, how strong is the effect? The Ministry of Health in Arequipa, Peru, in preparation for an insecticide application campaign, surveyed 7,959 of 12,069 (65.9%) households in the district of Mariano Melgar, Arequipa, Peru. 608 (7.6%) of surveyed households were infested with the vector. We carefully mapped the data from this survey, and the location of all streets in the district. We use a spatial statistic, Moran's I, adapted to a structured context, to assess separately the spatial auto-correlation within city blocks and across city blocks. We propose a simple test for statistical significance of the effects of streets on the statistic. We then perform a multivariate analysis to assess the strength of streets as barriers, controlling for co-factors. We find that decrease in the strength of the auto-correlation attributable to streets is similar to that due to an increase in distance of 40 meters. As the strong effect of streets on the distribution of *T. infestans* cannot be explained by known co-factors, these results strongly suggest that *T. infestans*'s dispersion is to some extent limited by streets. The dynamic of Chagas disease is directly linked to the spatial dynamic of the vector. Our results yield important implications for the understanding of these dynamics in urban areas. Of particular interest is the possibility to use the city blocks as units of prediction and control in further approaches.

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STATUS OF TSETSE-TRANSMITTED TRYPANOSOMIASES IN LIVESTOCK AND MAN IN THE MANAFWA-RIVER-CRESCENT DISTRICTS OF SOUTHEASTERN UGANDA

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A situation analysis of the tsetse-transmitted Trypanosomiasis problem in Uganda will update the GIS-based decision-support tool for reducing the impact of Tsetse-transmitted Trypanosomiases in livestock and man across agro-pastoral farming communities in Uganda. Mapped relationships derived from primary data (tsetse fly vector species and incidence of Trypanosomiases (nagana and sleeping sickness) from districts in Manafwa-Malaba river system in SE Uganda known to be affected by floods and landslides since 2007 shows that bovine Trypanosomiasis in all affected districts varies little between extended wet and dry seasons in September 2010 and January-February 2011. Nagana situation in September 2010 was overall for Manafwa at 16% (with Bugobero - 1%; Butiru - 25%; Bushiende - 7%; and Busiu - 38%); Mbale at 38%; Iganga at overall 4% (Ibulanku - 2%; Namungalwe - 3%; Nawandala - 0%; Nawaikoke - 2%) and Namutumba - 10% (Bulange - 5%-12%; Magada -12%). Nagana data for the above regions in 2011 showed the problem

is still heavy in the Manafwa (16%); slightly low in Mbale - 33%; Butalejja at Bunghazi -31% and Himutu - 8%). The survey was extended to Kumi - Mukongoro/Agaria area were we saw 10% infection with 8/14 being T. *vivax* 2010 survey found only biting flies in the area; Ngora - Kobuin/ Atoot had 4% Iganga-Ibulinaku had 3.8 % while Namutumba - Bulange had 10%. This data showed no reduced prevalence of nagana in districts bodering Manafwa river namely Manafwa, Busiu, Butalejja and Namutumba. Besides there is now an outbreak of sleeping sickness in Bukedea country where tsetsefly presence is now confirmed in previously un-infested communal grazing valleys in the heart of the district. This data will be related to the tsetse genetic mtDNA haplotype mapping and will be geo-processed. Reports will provide for a rational evidence-based protocol for managing Tsetse-transmitted Trypanosomiases (human and animal) in mid South-Eastern Uganda.

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EMPLOYING VALIDATED *LEISHMANIA* HIGH THROUGHPUT SCREENING ASSAYS TO IDENTIFY NOVEL ANTI-LEISHMANIAL CHEMOTYPES AND DIFFERENCES IN LIFE CYCLE CHEMOSENSITIVITIES

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We developed an automated, alamar blue-based, high throughput screening (HTS) assay for drug susceptibility of *Leishmania amazonensis* axenic amastigotes. We initially validated our screening system using the 1280 compound Library of Pharmacologically Active Compound (LOPAC) set. The assay performed robustly with average Z-factor and signal-to-background being 0.65±0.05 and 5.2±0.15, respectively. Our primary active rate for the LOPAC set using the axenic amastigote population was 3.1% at a screening concentration of 10 µM. We next used our screening assay to interrogate a diversity-based 220,335 compound library. The validated assay performed robustly with average Z-factor and signal-to-background of 0.43±0.12 and 4.6±0.1, respectively and a primary active rate of 1.7% at a 10 µM screening concentration. Comparing these statistics with those collected from a *Leishmania major* promastigote HTS drug susceptibility assay (using the same alamar blue format and screening concentrations), we found the primary active rates to be uniformly higher for the promastigote life cycle form. Specifically, screening the LOPAC set as well as a diversity-based compound library similar in size (i.e., 200,000 compounds) with nearly identical composition in the promastigote HTS assay yielded primary active rates of 10.5% and 8.9%, respectively, representing a 3-5-fold higher primary active rate than what we documented with the axenic amastigote assay. Interestingly, 1160 compounds exhibited >65% inhibition of growth against both *Leishmania* life cycles. Preliminary structural clustering of these chemotypes indicated 167 common structural clusters (ranging in size from 2 to 11) with the remaining 657 compounds being classified as singletons. We will highlight and discuss potential differences in the chemosensitivities of the *Leishmania* parasite life cycle forms and the importance of compound library selection in the search for novel anti-leishmanial chemotypes.

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TRYPANOSOMA CRUZI: SUSCEPTIBILITY OF CULTURED EPIMASTIGOTES TO SINGLE AND PAIRED TREATMENTS OF RECOMBINANT ANTIMICROBIAL PEPTIDES EXPRESSED FROM THE *RHODOCOCCUS RHODNII* SYMBIONT OF THE *RHODNIUS PROLIXUS* CHAGAS DISEASE VECTOR

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Chagas disease, or American trypanosomiasis, results from infection with the protozoan parasite, *Trypanosoma cruzi,* and can result in chronic disease characterized by cardiac and gastrointestinal dysfunction. *T. cruzi* is vectored by domiciliary triatomine insects that deliver the parasite to humans during blood meals by deposition of infected feces. Chagas control programs have focused on elimination of the vectors through pesticide applications and have been effective in the short term; however, human health concerns, emerging pesticide resistance, and overall cost of the efforts hamper long term sustainability. Paratransgenesis is an alternative method of Chagas control that functions by interrupting the cycle of parasite transmission. Bacterial symbionts of the *T. cruzi*-carrying vectors are transformed with expression plasmids whose products are toxic to the parasite. These symbionts are delivered to the vector by simulated coprophagy and reside in the hindgut near the infectious trypomastigotes. Previous *in vivo* tests using the *Rhodococcus rhodnii* symbiont of the *R. prolixus* vector expressing the cecropin A anti-microbial peptide (AMP) resulted in the complete clearance of infective *T. cruzi* in ~70% of the vectors and a decrease in parasite titers for the remainder. *In vitro* toxicity testing of multiple AMP's against *T. cruzi* identified candidate AMP's that exhibited additive and synergistic lethal concentrations. The DNA sequences for these AMP's were cloned into expression shuttle vectors and transformed into *R. rhodnii*. Media was collected and cell lysates prepared from clones whose expression was confirmed by Western blot and ELISA. Treatment of *T. cruzi* in culture with AMP-positive media and lysates for 96 hours resulted in parasite killing as measured by reduction in normal cell density changes at 600 nm, and microscopic examination of live cell numbers with Calcein-AM. Media from AMP transformants was less toxic to *T. cruzi* than the corresponding cell lysates which showed substantially greater toxicity when combined in pair-wise treatments.

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OPTIMIZING INHIBITORS OF METHIONYL-TRNA SYNTHETASE FOR TREATING LATE-STAGE AFRICAN TRYPANOSOMIASIS

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Better drugs are desperately needed to treat human African trypanosomiasis, particularly for late-stage disease when the *Trypanosoma brucei* parasites have invaded the central nervous system (CNS). This poses a particular challenge in drug development as the blood brain barrier (BBB) effectively excludes most small molecules from attaining significant levels in the CNS. In previously published work, we described inhibitors of the *Trypanosoma brucei* methionyl-tRNA synthetase with EC_{50} values as low as 4 nM on *T. brucei* cultures and that demonstrated potent activity in the murine model of acute *T. brucei* infection. Unfortunately, the described quinolone compounds were observed to have poor permeability characteristics in the MDR1-MDCKII *in vitro* model of the BBB. Variations of the scaffold were synthesized and new compounds containing a urea core were discovered with excellent permeability properties in the MDR1-MDCK11 model. In addition, the new urea compounds have greater selectivity between the trypanosome methionyl-tRNA synthetase and the human mitochondrial tRNA synthetase (~200:1) in comparison to the quinolone compounds (~5:1). However, the urea compounds have

higher EC50 values (~150 nM) against *T. brucei* cultures, thus more potent analogs are needed. To aid the compound design process, crystal structures of the *T. brucei* methionyl-tRNA synthetase have been solved in complex with several urea compounds to define the binding mode and opportunities for improving affinity to the enzyme. We will report on progress on improving anti-*T. brucei* activity while optimizing CNS permeability and metabolic stability of new lead compounds.

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CO-INFECTION OF KALA-ZAR AND FLAVIVIRUSES: A CASE REPORT OF A PATIENT FROM NORTHERN KENYA WHO WAS SEROLOGICAL POSITIVE FOR KALA-ZAR AND FLAVIVIRUSES, 2010

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In mid September 2010, a 50 year old male patient from Wajir town, North-eastern province, Kenya was seen at a private clinic in Nairobi with complaints of fever, epistaxis, joint pain and myalgia. He had been unwell since mid April 2010 and reported having had jaundice, which subsided after one month, and marked weight loss. Clinical laboratory investigations showed pancytopaenia with mild deranged liver function tests. A blood sample was collected after clinical tests and sent to the viral haemorrhagic fever laboratory at Kenya Medical Research Institute (KEMRI) for arbovirus tests. IgM, IgG ELISA and RT-PCR tests were conducted for Yellow fever (YFV), Dengue (DEN), West Nile (WNV), Chikungunya (CHIK), Rift Valley fever (RVFV) and Crimean Congo Haemorrhagic fever (CCHF) viruses. Both IgM and IgG ELISA tests were positive for Flaviviruses but negative for other arboviruses tested, suggesting that the patient could have possibly had a prior exposure to one of these flaviviruses. RT-PCR was negative for both group primers for Flaviviruses and specific primers for YFV, DEN and WNV. Later the same month, the patient was admitted at Kenyatta National Hospital with a fever of 39.9°C and massive hepato-splenomegally. Further tests were conducted at KEMRI, to rule out Kala-zar, using four different rapid detection kits: Diamed IT-Leish Kit, Signal KA kit, rK39 and InBios Kalazar detect kit. The sample was positive for Kala-zar by all the kits. Ministry of Public Health and Sanitation sent a team to Wajir County to review the hospital records and establish the burden of Kala-zar. A total of 600 patient records from Wajir met the case definition, of which 237 patient records had been positive by rK39. The results show that Kala-zar is endemic in Wajir County. The positive results for flaviviruses indicate the possibility co-circulation of Kala-zar with these viruses, such as YFV, DEN and WNV. Systematic studies need to be conducted to determine sero-prevalence levels and factors associated with Kala-zar and arboviruses.

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THE WHO THRESHOLD OF 50% SCHISTOSOMIASIS PREVALENCE AMONG SCHOOL-AGED CHILDREN TO EXPAND PRAZIQUANTEL MDA TO ADULTS IS NOT USEFUL IN CENTRAL NIGERIA

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WHO guidelines for preventive chemotherapy (PCT) call for praziquantel (PZQ) treatment of adults, in addition to treatment of school aged children, where schistosomiasis (SCH) prevalence in school-aged children is ≥50%. The purpose of this study was to ascertain the value of the 50% threshold in predicting higher infection rates in adults, and so justify the additional cost of expanded PZQ treatments. We evaluated urinary SCH prevalence in adults in hyperendemic communities (where ≥50% in children were heme dipstick positive), compared to SCH prevalence in adults in mesoendemic communities (where heme dipstick positivity ranged from 20-49% in children). The study was conducted in Plateau and Nasarawa states of north-central Nigeria. From baseline mapping of SCH among school-aged children in 2008, 7 hyperendemic and 12 mesoendemic communities across 4 districts were selected for evaluation. The prevalence of hematuria (reagent stick) and the presence and intensity of *Schistosoma hematobium* eggs in urine was determined among adults aged 20 years and older from randomly selected households in each community. A total of 1,164 adults were examined out of 1,287 registered; 505 in hyperendemic communities (where mean hematuria among children in 2008 was 70.4%) and 659 in mesoendemic communities (where mean hematuria among children in 2008 was 26.6%). The prevalence of hematuria was similar among adults in hyperendemic communities (18.2%, 95% CI 11-25%) and mesoendemic communities (17.8%, 95% CI 8-27%). Similarly, the prevalence of infection was 21.0% (11-31%) in hyperendemic communities and 17.0% (7-27%) among the mesoendemic communities. The prevalence of intense infections (defined as egg density of ≥10 eggs/5 ml) was 1.2% (0.1-2.3%) and did not differ by community group. We concluded that in this setting there was no evidence to implement an expanded treatment program that would include adults only where the prevalence of micro-hematuria in school-age children was ≥50%.

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TREATMENT OF SCHISTOSOMIASIS IN INFANTS AND PRESCHOOL-CHILDREN WITH PRAZIQUANTEL: CORRECT DOSE, SIDE-EFFECTS AND CURE RATES

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In large-scale interventions for control of schistosomiasis, use of the World Health Organization dose pole is favoured for mass-drug administration of praziquantel to school-aged children and adults. Application of this simple tool has enabled pragmatic tablet dosing using patient height as a proxy for body weight, allowing control programmes to expand into resource-poor settings. New evidence advocates the immediate inclusion of preschool aged children (≤5 year olds), also at high risk for disease and morbidity, in future control campaigns; therefore, the current WHO pole needs updating. Height and weight data were measured during several epidemiological surveys conducted in Angola (N=1067), Mali (N=405), Uganda (N=3303), Sudan (N=137), Zanzibar (N=470) and Zimbabwe (N=104) to establish and validate an extended PZQ dose pole, which now includes two new height-intervals: 60-84cm for ½ tablet and 84-99cm for ¾ tablet divisions. Anthropometric data from other African countries (Demographic Health Surveys) are now also available and will be analysed in the near future. Treatment has been given to different child cohorts and results show that while treatment cure rates can vary significantly between cohorts (25-100%), side-effects tend to be mild and transient. Theoretical application of the updated dose pole results in >95% of children receiving an acceptable dose (30-60 mg/kg). Using this pole, we suggest that mass-drug administration can be better optimized, streamlining general treatment to reduce drug wastage which could lead to significant programmatic savings and allocation of treatments to younger children with minimal additional cost.

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PREVALENCE AND INTENSITY OF *SCHISTOSOMA* SPP TWO YEARS AFTER A PRAZIQUANTEL TREATMENT AMONG SCHOOL-AGE CHILDREN FROM A RURAL VILLAGE FROM AN IRRIGATION SCHEME SUBJECTED TO MULTIPLE TREATMENTS WITH PRAZIQUANTEL, MALI

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Schistosomiasis remains a major public health problem in developing countries with praziquantel (PQZ) being the only treatment available for prolonged use to prevent associated morbidity. The central issue remains about the optimal interval between PQZ treatment rounds to achieve significant long term decrease in worm loads. We evaluated the impact of a single dose of PQZ treatment on the prevalence and infection intensity of *Schistosoma mansoni* and *S. haematobium* in a rice irrigated village in Mali. Two cross-sectional parasitological surveys of children (6-14 years old) were carried out in 2005 and 2007 in a single village in Mali. Stool and urine samples were examined for *S. mansoni* and *S. haematobium* eggs. Difference in prevalence and infection intensity between the surveys was tested adjusted for age and sex by logistic, negative binominal (NB) and zero-inflated negative binominal (ZINB) modelling. 1948 single *S. mansoni* parasite were genotyped. At 2 years post-treatment, the overall prevalence of *S. mansoni* infection was stable, 93% and 88% [OR 0.55, CI95 0.26-1.10], while *S. haematobium* infection fell significantly from 74.5% to 28.0% [OR 0.12, CI95 0.07-0.20]. Geometric means of *S. mansoni* and *S. haematobium* infections decreased significantly from 179 to 83 eggs/gram of faeces [egg count ratio (ECR) 0.58; CI95 0.42-0.78] and 12.3 to 1.8 eggs/10 ml urine [ECR 0.074, CI95 0.044-0.127]. The proportion of children with heavy infections decreased significantly from 42% to 26% for *S. mansoni* and 26% to 0.9% for *S. haematobium*. *S. mansoni* molecular epidemiology identified closely related strains. In conclusion, the ZINB model was effective for the analysing egg count data due to excess zero observations. Praziquantel appeared to have a long term effect on *S. haematobium* but not on *S. mansoni* thought this might also suggest species-specific differences in praziquantel treatment. Sufficient reduction of schistosomiasis infection was not attained and requires additional control measures specific to the 'Office du Niger' irrigation scheme to synergise chemotherapy.

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TRANSCRIPTIONAL RESPONSES OF *SCHISTOSOMA JAPONICUM* EXPOSED *IN VIVO* TO SUB-LETHAL DOSAGES OF PRAZIQUANTEL

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The central reliance of praziquantel (PZQ) for the treatment and control of schistosomiasis is a concern from a public health perspective. PZQ displays a bimodal spectrum of activity being active only against very young schistosomules and the sexually mature blood flukes. Adult schistosomes exposed to PZQ respond with muscle contraction, paralysis, membrane depolarization and the influx of extracellular Ca^{2+}. Molecular characterisations of Ca^{2+} homeostasis, while providing some insights on drug resistance, have been based on initial *in vitro* observations only. Despite these useful studies the precise identity of the molecular targets and mechanisms of detoxifying PZQ are unknown. We have used a murine model to administer *in vivo* sub-lethal dosages of PZQ to adult *Schistosoma japonicum*. Differential gene expression of parasites was followed between 30min and 24h post-PZQ administration, using a whole transcriptome microarray platform. Differential gene expression was considered separately for male and female worms. In males up-regulated genes were associated initially with functions such as "Tegument/Muscle Repair" and "Lipid/Ion Regulation", while later responses included "Drug Resistance" and "Ion Regulation". In contrast, in females, a different sub-set of genes were initially up-regulated including those involved with "Ca^{2+} Regulation" and "Drug Resistance". Genes associated with "Detoxification" and "Pathogen Defense" functions were more prominently upregulated during the later response of female worms to drug treatment. The unique combination of chemotherapy together with the host immune response, provides a more biologically relevant insight into the effects of PZQ on adult schistosomes. Following on from the microarray analysis, we used qPCR to validate a sub-set of genes with either putative drug resistance/detoxification roles or Ca^{2+}-dependant/ modulatory functions. The functional importance of these genes for parasite survival after PZQ treatment was corroborated using RNAi and *in vitro* PZQ assays.

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TOWARD THE ASSESSMENT OF PHYTOCHELATIN SYNTHASE AS A DRUG TARGET FOR SCHISTOSOMIASIS

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Schistosomiasis is a parasitic disease caused by blood flukes of the genus *Schistosoma*, responsible for more than 280,000 deaths annually. The treatment of the disease relies on a single drug, praziquantel. Because it is a cost-effective drug, it has been disseminated through control programs; hence, it is likely that resistance of the parasite to the drug emerge. Therefore, there is an urgent need to identify new targets and drugs for schistosomiasis treatment. We are currently investigating the potential of phytochelatin synthase (PCS) as a drug target in *S. mansoni*. This enzyme is of particular interest since humans do not have a PCS gene in their genome. PCS is a cysteine protease-like enzyme that catalyzes the production of glutathione-derived peptides, the phytochelatins (PCs), with a structure (γGlu-Cys)nGly (n=2-11). These peptides are known to be involved in heavy metal detoxification and accumulation (Pal and Rai 2009). Initial analyses of *S. mansoni* PCS indicated that it protects yeast from metal toxicity and that its expression in cultured worms is increased in response to the presence of metals (Ray and Williams 2011). To assess the function of this protein in *S. mansoni*, studies on the recombinant enzyme have been carried out. Recent work with the purified recombinant *S. mansoni* enzyme showed evidence for the production *in vitro* of PCs (γGlu-Cys)nGly, with n=2-7) from glutathione. Enzyme activity was measured by fractionation of PCs using HPLC, identification by MALDI-TOF, and quantification by derivatization of the PCs with Ellman's reagent. Interestingly, using glutathione-S-bimane as a substrate, *S. mansoni* PCS is capable of cleaving the glycine residue yielding the corresponding γGlu-Cys-S-conjugate. These data attest to the role of PCS in potential detoxification pathways, for both heavy metal scavenging and xenobiotic-glutathione conjugate degradation, making this enzyme likely to be necessary for the parasite, particularly in stress conditions. We are currently developing a biochemical assay that could be used in a high throughput manner that would allow us to screen for inhibitors. Identification of PCS inhibitors will help us to understand the function of the protein in the parasite and will be used to assess its potential as a drug target in a mouse model of infection

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SCHISTOSOMA MANSONI HISTONE-MODIFYING ENZYMES AS DRUG TARGETS

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Enzymes that modify histones (HME) are under increasing scrutiny as therapeutic targets in a number of pathologies, ranging from cancer to parasitic diseases. In particular, inhibitors of histone deacetylases (HDACi) induce cell cycle arrest and/or apoptosis in cancer cells. Treatment of schistosomula or adult worms with HDACi induces the death of both larval (schistosomula) and adult worms and this is preceded in the larvae by the induction of apoptosis as measured by TUNEL staining and the increase in the activity of caspase 3/7. Moreover, such treatments induce a rapid increase in the general level of histone acetylation, particularly of H4. This in turn correlates with the overexpression of certain genes, including those encoding caspases 3 and 7. Finally, qChIP analysis shows that the proximal promoters of both these genes show hyperacetylation of histone H4 after HDACi treatment. These results led us to consider schistosome HDACs, as well as other HMEs, as promising targets for the development of new drugs against schistosomiasis. To this end, a project (SEtTReND) supported by funding from the European Commission has been initiated with the aim of developing specific inhibitors against selected schistosome HMEs that could be candidates as lead compounds for drug development. All HMEs encoded in the *S. mansoni* genome involved in histone acetylation/deacetylation and methylation/demethylation have been identified. Using a phenotypic screen we have shown that inhibitors of all these enzyme classes induced apoptosis and death in schistosomula. Among the schistosome HMEs chosen for further study, SmHDAC8 is particularly promising. Its catalytic domain is more divergent from the human orthologue than are those of other schistosome HDACs and its validity as a therapeutic target was confirmed by RNAi. A combination of high-throughput and in silico screening is being applied to identify potential specific inhibitors of SmHDAC8. In parallel, other HMEs are also being investigated as potential targets.

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CHARACTERIZATION OF FARNESYL DIPHOSPHATE SYNTHASE AND GERANYLGERANYL DIPHOSPHATE SYNTHASE IN *SCHISTOSOMA MANSONI* AND THEIR ROLE AS POTENTIAL DRUG TARGETS

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Schistosomiasis affects over 260 million people worldwide with over 200,000 deaths annually. There is currently only one drug available for disease treatment, praziquantel. We report here that *Schistosoma mansoni* farnesyl diphosphate (FPP) synthase (SmFPPS) and geranylgeranyl diphosphate synthase (SmGGPPS), essential enzymes in many eukaryotes involved in protein prenylation and the generation of sterols and non-sterol products of mevalonate, could serve as drug targets for the treatment of schistosomiasis. In humans, FPPS is a target for the bisphosphonate drugs widely used in bone resorption therapy. Validation of FPPS and GGPPS as drug targets may allow the repositioning of bisphosphonates for schistosomiasis treatment. SmFPPS and SmGGPPS have 35% identity to human FPPS and 53% identity to human GGPPS, respectively. We successfully expressed active, recombinant SmFPPS and SmGGPPS. Recombinant SmFPPS was found to be a soluble 44.2 kDa protein while SmGGPPS was a 38.3 kDa soluble protein. Characterization of the substrate utilization of the two enzymes showed that, unlike the human enzymes, which display strict substrate specificity, both worm enzymes were able to couple isopentenyl PP with three allylic acceptors (dimethylallyl diphosphate, geranyl diphosphate, and FPP). This indicates that the schistosome enzymes have overlapping substrate specificities, making their actions appear to be redundant. Against SmFPPS, several bisphosphonates had IC50s in the low nanomolar range; these inhibitors had significantly less activity against SmGGPPS. While hydrophilic bisphosphonates had no activity against cultured adult parasites, a lipophilic bisphosphonate at 50 µM was active against ex vivo adult worms with worm death occurring over 4-7 days.These results indicate that FPPS and GGPPS could be important targets in the search for new drugs for the treatment for schistosomiasis.

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SINGLE NUCLEOTIDE POLYMORPHISM-BASED SELECTIONS IN THE ß-TUBULIN GENE OF *ONCHOCERCA VOLVULUS*: A NEW STEP IN FILLING THE GAP OF THE POSSIBLE IVERMECTIN FAILURE

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The control of onchocerciasis or river blindness with ivermectin (IVM) has been a great success until now, so that in certain foci its elimination was found to be feasible. However, after more than 21 years of IVM repeated treatment, the disease still persists in many endemic countries. Though sub-optimal responses and genetic changes have been reported in *Onchocerca volvulus* populations under high IVM pressure, unequivocal evidence of resistance has yet to be established. This situation must therefore be urgently clarified to preserve the achievements of onchocerciasis control programs. In this study, *O. volvulus* adult worms were collected from the same patients, before IVM exposure and following three years of annual or three-monthly treatment at 150 µg/kg or 800 µg/kg. Four single nucleotide polymorphisms (SNPs) occurring in the β-tubulin gene of these parasites were investigated. We found multiple nucleotide changes in *O. volvulus* β-tubulin gene associated with the dose and the annual frequency of IVM. Among the SNPs investigated, three showed a high level of selection and nonrandom allelic associations after treatment. Therefore, they may be relevant markers to follow selection for IVM resistance in the field. These results strengthen the warning that selection for IVM resistance could emerge in some *O. volvulus* populations.

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A LATERALLY TRANSFERRED FERROCHELATASE GENE IS FUNCTIONAL AND ESSENTIAL IN FILARIAL NEMATODE PARASITES

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Species in the phylum Nematoda lack a heme biosynthetic pathway and require extraneous heme. Many filarial nematodes contain an obligate endosymbiont, *Wolbachia,* which has a functional heme biosynthesis pathway. Sequencing of the human filarial nematode *Brugia malayi* revealed a genomic ferrochelatase (BmFc) gene, the terminal step in heme biosynthesis. The BmFc gene contains 9 exons spanning ~ 4.5

kb and includes a mitochondrial-targeting domain. The ferrochelatase is functional based upon enzyme assay, complementation to an *E. coli* hemH$^-$ mutant, inhibitor studies with a ferrochelatase-specific inhibitor in *B. malayi* and as a transgene in *Caenorhabditis elegans*. RNAi inhibition experiments also provide evidence that BmFc is functional. While the mitochondrial targeting domain is required for mitochondrial location, it is not required for enzyme activity. FISH reveals the BmFc gene is almost universally expressed in both male and females tissues, except for female late-stage embryos and male late stage sperm cells. Orthologues have been identified in several other filaria, as well as from non-*Wolbachia* containing species and a non-filarial nematode. Phylogenetics suggests a non-Wolbachial, but α-proteobacterial, origin with the lateral transfer acquisition predating the split of the Rhabditida into the Spirurina and Rhabtinia clades. This is the first reported functional LGT gene in animal or human filarial nematodes and its requirement for worm viability suggests it could play a role in the symbiotic relationship between the filarial nematode host and its symbiont and be a potential target for drug discovery against filariasis.

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CLONING AND CHARACTERIZATION OF THE TREHALOSE-6-PHOSPHATE PHOSPHATASE FROM *BRUGIA MALAYI*, A CANDIDATE ANTIFILARIAL DRUG TARGET

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Approximately 120 million people are infected by either *Brugia malayi* or *Wuchereria bancrofti*, the parasitic nematodes responsible for lymphatic filariasis. Although there are several drugs available to treat this disease, there remains a need for additional pharmacological therapies. A draft sequence of the *B. malayi* genome is available and it shares significant similarity to the genome of the well-characterized free-living nematode *Caenorhabditis elegans,* as reported previously. The wealth of information about and the robust genetic tools of *C. elegans* can be used to aid the search for new antifilarial drug targets. One study ranked approximately 600 predicted drug targets from *B. malayi* without human homologs based, in part, on the strength of the RNAi knockdown phenotype in *C. elegans*, as reported previously.We focused our studies on one gene ranked in the top 40 of the predicted targets, the homolog of the *C. elegans gob-1* (gut-obstructed) gene. Consistent with its high ranking in this list, this gene is both essential in *C. elegans* and there is no homolog in the human genome. This gene encodes a trehalose-6-phosphate phosphatase and is required for the biosynthesis of trehalose (as reported previously). We cloned the *B. malayi gob-1* gene (Bm_GOB-1) and expressed it in *E. coli.* We purified Bm_GOB-1 and biochemically characterized its phosphatase activity. Using *C. elegans* we confirmed the observation that the accumulation of trehalose-6-phosphate (T6-P), rather than the reduction of trehalose, is likely responsible for the observed lethality (as reported previously). We are currently examining biochemically whether T-6P inhibits the activity of *B. malayi* hexokinases in an effort to understand the mechanism of the T-6P toxicity. Further characterization of both Bm_GOB-1 and the T6-P toxicity may aid in the development of new antifilarial therapies.

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TARGETING THE *WOLBACHIA* CELL DIVISION PROTEIN FTSZ AS A NEW APPROACH TO ANTIFILARIAL THERAPY

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The use of antibiotics targeting the obligate bacterial endosymbiont *Wolbachia* of filarial parasites has been validated as an approach to controlling filarial infection in animals and humans. The availability of genomic sequences for the *Wolbachia* present in the human filarial parasite *Brugia malayi* enables genome-wide searching for new potential drug targets. FtsZ is such a target protein as it is essential for bacterial cell and absent from humans. In the present study, we have cloned, expressed and purified *Wolbachia* and E. coli FtsZ protein. We determined that *Wolbachia* FtsZ protein possesses the GTPase activity using the spectrophotometric coupled enzymatic assay. We demonstrate that the *Wolbachia* FtsZ GTPase activity was inhibited by berberine and validated the berberine's antibacterial effect using E. coli as a model organism. A library of naphthalene-, quinoline- and biphenyl-based compounds, which was constructed using Ugi multicomponent reaction chemistry, was examined for the discovery of antagonists of E. coli and *Wolbachia* FtsZ. From screening efforts, the (6-{butylcarbamoyl-[(aryl)-(butylcarbonyl)-amino]-methyl})-naphthen-2-ol scaffold emerged as a potent antagonist of both E. coli and *Wolbachia* FtsZ. Interestingly, from structure-activity relationship studies it appears that modification of the aryl substituent on the scaffold may afford selectivity for *Wolbachia* FtsZ. Additional compounds are currently being prepared to examine this possibility. Our results will facilitate the discovery of selective inhibitors of FtsZ as a novel anti-symbiotic approach to controlling filarial infection.

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HSP90 AS A TARGET IN FILARIAL NEMATODES

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In order to identify novel inhibitors of Hsp90 in filarial worms, we adapted a fluorescence polarization assay that was originally designed for screening inhibitors of Hsp90 in tumor cells. The assay relies upon the ability of small molecules to inhibit the binding of fluorescently labelled geldanamycin to Hsp90 and has the advantage of using extracts of worms rather than requiring recombinant protein. The assay was validated using known inhibitors of Hsp90 that compete with geldanamycin for binding to Hsp90, including members of the synthetic purine-scaffold series, and was sufficiently sensitive to differentiate between binding of PU-scaffold compounds to human and *Brugia* Hsp90. The assay was then used to screen a focused kinase library and identified several hits, two of which were tested on adult worms *in vitro*. One of these molecules has a significant effect on adult worms *in vitro* and thus provides a scaffold for further structure based drug activity studies.

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CLONING AND OVEREXPRESSED STUDIES ON HUMAN LUNG MAST CELL RECOMBINANT CARBOXYPEPTIDASE A IN *SACCHAROMYCES CEREVISIAE*

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Mast cell carboxypeptidase A (MC-CPA) is a highly conserved secretory granule protease. It specifically catalyzes the hydrolysis of the peptide bond adjacent to the C-terminal end of a polypeptide chain. Little is known about the function of this enzyme. It has been established however, its ability to cleave the substance angiotensin I into angiotensin II. This suggests that the human mast cell carboxypeptidase A might play a role in the hypertension disease. Normally, the biological actions of proteases are controlled by specific interactions with proteinaceous inhibitors. So far, however, only a few protein inhibitors have been identified for this type metallocarbopeptidases. To shed more light to the function of Human Mast Cell Carboxypeptidase A (hMCCPA) to screen for novel inhibitors for this enzyme we cloned and overexpressed the gene in *E. coli.* The recombinant protein however was expressed in the form of inclusion bodies. To overcome this problem we cloned the gene in *Saccharomyces cerevisiae*. The aim of this project was to produce a soluble active form of CPA in *Saccharomyces cerevisiae.* Gene cloning

was carried out using the vector pYES2 (Invitrogen). The results showed that the gene has been successfully overexpressed in the yeast. Our study also optimized the growth conditions to produce soluble recombinant CPA. The overexpressed soluble CPA was then purified using the one step purification technique.

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EXPRESSION OF PUTATIVE MOLTING-ASSOCIATED GENES IN POST-INFECTIVE FILARIAL L3

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Nematode molting is a complex process that requires synthesis of new cuticle coordinated with other developmental changes plus shedding of the old cuticle. Prior studies have shown that tetracyclines inhibit molting of *Brugia malayi in vitro* and *in vivo*. The *B. malayi* genome contains orthologues for many genes that are required for molting in *Caenorhabditis elegans* based on RNAi results. The purpose of this study was to compare expression profiles of orthologues of putative molting genes in pre- and post-infective larvae of *B. malayi*. L3 were obtained from mosquitoes (vL3) and post infective larvae were recovered from jirds 3 and 6 days after ip injection. We used qRT-PCR to assess relative expression levels for 56 putative molting genes. 73 and 66% of these genes were differentially expressed on day 3 and day 6 (>2 fold change relative to vL3). 13 were up-regulated and 28 were down-regulated on day 3, and 21 were up-regulated and 16 down-regulated on day 6. 57% of these genes were also differentially expressed (25 were up-regulated and 7down-regulated) in day 6 larvae compared to day 3 larvae. Up-regulated genes in post-infective L3 encode homologues of proteases (*nas-37*, *nas-36*), protease inhibitors (*mlt-11*), peroxidase (*bli-3*), DNA binding and sterol sensing domains (*nhr-23, ptr4, ptr23*), extracellular matrix (*noah-2*), signaling and novel genes. Some genes required for molting may also be essential for growth and development. Three days of ip doxycycline (25 mg/kg) had no significant effect on expression of putative molting genes in day 3 larvae. Additional studies are in progress to assess effects of longer exposure to Doxy on gene expression prior to the L4 molt. This research has provided new information on changes in expression of putative molting genes in post-infective *B. malayi* L3. Additional studies will be needed to verify whether these genes are essential for molting in filarial worms.

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ASSESSMENT OF THE VIABILITY OF FILARIAL PARASITES USING MOLECULAR MARKERS

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Many major investigative activities, such as assessing the effects of macrofilariacides and the immune system on these complicated parasites, depends most commonly on assessment of their morphological status. The morphological assessment of the viability and the stages of degeneration of mature filarial worms however, is a difficult and often very subjective process. The use of in situ molecular markers by immunocytochemistry has been used by a number of investigators often with reagents that are directed against mammalian antigens, rather than using those that have been clearly defined as nematode constituents. We have identified markers that appear to have homologous presence and functions in both mammals and nematodes. These immunocytochemical reagents directed against putative nematode components of cellular metabolism and replication have been used to reflect the effects of ivermectin on adult Onchocerca volvulus. The presence of these markers can be quantitated and provide more objective data as to the status of adult worms than has been previously used. The results from the use of these markers suggests that the long term use of ivermectin has a general depressive effects on the health and in all likelihood the longevity of this worm. This approach to assessing worm viability and degenerative status is believed to be suitable for general use and allows this important assessment activity to be carried out by a wider range of scientists than only those with extensive parasitological knowledge.

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DIFFERENTIAL EXPRESSION OF CYS-LOOP LIGAND-GATED ION CHANNEL GENES IN *BRUGIA MALAYI* ADULT WORMS AND MICROFILARIAE

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Nematode cys-loop ligand gated ion channels (CLGIC) are important targets for anthelmintics such as macrocyclic lactones (MLs) and nicotinic agonists. Different parasite species and stages within species vary with respect to sensitivity to drugs that target CLGIC. For example, MLs that target glutamate-gated chloride receptors (GluCl) are highly effective against some gastrointestinal nematode species and filarial L1 larvae (microfilariae, or Mf), but they are less effective against filarial adult worms. Drug sensitivity may be related to CLGIC expression levels. Therefore, we used qRT-PCR to assess relative transcription levels for 32 CLGIC genes in Mf and adult worms of the filarial nematode *Brugia malayi*. These genes encode different classes of GLGIC including GluCl, nicotinic acetylcholine receptors (nAChR), and gamma amino butyric acid (GABA) receptors. Interestingly, transcription levels of GluCl subunits (Bma-AVR-14 A and B) were highest in females, intermediate in Mf, and lowest in males. Most genes encoding nAChR had higher expression in males than in Mf or females, while most genes encoding orphan group channels were most highly expressed Mf. These results show that expression of GLGIC genes varies between life cycle stages and sexes in filarial worms. These differences may reflect stage-specific differences in neurobiology and explain in part the variable sensitivity of filarial stages to anthemintic compounds that target neurotransmission.

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A COMPARATIVE ANALYSIS OF NEMATODE EXCRETORY-SECRETORY PRODUCTS

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Excretory-secretory products (ESP) from parasitic nematodes are thought to be involved in a series of processes that determine their fate, to succeed or fail in host infection. Although the identification of these products has often been limited by the typically low amount of recovered material from *in vitro* incubations, developments in mass spectrometry-based protein identification, along with genomic and transcriptomic sequencing platforms, offer a new way to overcome this limitation and to investigate the roles of components of ESP at the host-parasite interface. To gain a deeper understanding of how parasitic nematodes survive, and the multiple strategies that they may employ to adapt to each particular host and niche therein, we initiated a comparative analysis of ESP from several parasitic species, including *Brugia malayi*, *Meloidogyne incognita* and *Heligmosomoides polygyrus*, as well as the free-living species *Caenorhabditis elegans*. ESP were collected and analyzed through 1D-SDS PAGE and LC-MS/MS. Strategies for MS-to-peptide assignation included database searches on either protein models datasets assessed from their respective genome projects or the deduction of 6 open reading frames from next-generation sequencing transcriptomic assemblies. Comparisons were carried out in terms of protein homologues identified as well as

their functional annotation inferred using bioinformatic tools. Differences in the suite of ESP from parasitic and non-parasitic species reveal specific patterns that may be associated with different niches and host locations.

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THE ROLE OF MELATONIN IN REGULATING NOCTURNAL PERIODICITY OF AVIAN MICROFILARIAE (*CHANDLERELLA QUISCALI*) WITHIN ITS NORMAL HOST, THE COMMON GRACKLE

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Lymphatic filariasis is a debilitating mosquito-borne disease affecting millions of people throughout the tropics. It is caused by filarial nematodes (*Wuchereria bancrofti* and *Brugia malayi*) that inhabit the lymphatic system. To perpetuate their life cycle, female worms produce millions of microfilariae (mf) that enter the blood stream, in the hopes that some will be ingested by mosquito vectors. Throughout most of their range, *W. bancrofti* and *B. malayi* exhibit nocturnal periodicity - i.e., mf only appear in peripheral blood at night. During the day, mf are sequestered in the alveolar capillaries of the lungs and are virtually absent from the peripheral blood. The cue or "pacemaker" responsible for synchronizing mf with the circadian rhythms of their human hosts has never been elucidated. We hypothesize that the pacemaker may be melatonin, a bioactive amine that is secreted from the pineal gland at a regular circadian periodicity. To test this, we used a locally-available avian system - i.e., *Chandlerella quiscali* mf in the Common Grackle. To determine the pattern of mf periodicity in this system, venous blood was taken every 26 hours from 7 microfilaremic grackles. Despite a wide range of mf intensities, the patterns of nocturnal periodicity were similar. Microfilaremias started at 2200h, peaked at 0200 to 0400h and subsided at 0600h. To determine if melatonin would cause mf to appear in peripheral blood, exogenous melatonin was injected into infected grackles during mid-afternoon when they are not microfilaremic. One bird received saline (control) and two received melatonin. The control bird remained amicrofilaremic. A high dose of melatonin (100ng) provoked an earlier appearance of mf than did a low dose (25ng), but at the end of the 3 hour post-treatment observation period, the low dose produced a higher microfilaremia, closer to that of a normal nighttime microfilaremia. Studying nocturnal periodicity of mf from the perspective of hormonal regulation of circadian rhythms may lead to a new approach to block transmission of lymphatic filariasis.

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IN VIVO DUAL TRANSCRIPTOME ANALYSIS OF FILARIAL WORM-MOSQUITO INTERACTIONS

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Filarial worms that cause lymphatic filariasis have a complex life cycle involving both human and mosquito hosts. Within mosquito tissues, nematodes undergo life cycle changes from microfilariae (mf) to infective-stage larvae (L3). Although critical for transmission and completion of the life cycle, molecular processes underlying parasite development in, and interaction with, the mosquito host tissue remain largely uncharacterized. In this study, we simultaneously profiled both the parasite and the host transcriptome over the course of parasite development from mf to L3. *Aedes aegypti* thoracic tissues infected with *Brugia malayi* were collected, in replicate, for 8 consecutive days at 24-hr intervals, and the *in vivo* dual transcriptomes were analyzed using RNA-seq. Tissue samples from mosquitoes fed on uninfected blood were analyzed in parallel. In addition, we evaluated the parasite-host interactome using a strain of *A. aegypti* that fails to support *B. malayi* development. Parasite transcripts ranged from 0.1 to 9.0% of the total transcripts recovered from thoracic tissues during the course of parasite development, but because our dataset is comprised of ~1 billion reads that mapped to the two reference genomes, this experimental approach provides an unprecedented view into parasite development within the mosquito host. In addition, the use of longitudinal and cross-sectional comparisons, both within and across the two organisms, enable a unique analysis of parasite-host interaction involved in this symbiotic relationship.

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THE COMPLETE MITOCHONDRIAL GENOME SEQUENCE OF FILARIAL NEMATODE *WUCHERERIA BANCROFTI*

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Wuchereria bancrofti (Wb) is the primary causative agent of lymphatic filariasis (LF), a deforming and debilitating disease estimated to affect 120 million people in 83 countries. Despite constituting a major public health problem in many tropical and subtropical regions, this mosquito-borne parasitic nematode remains poorly understood with respect to its mitochondrial (mt) genome sequence. To address this knowledge gap, the complete mt genome of Wb was sequenced following amplification and cloning of 15 overlapping mt fragments from a Papua New Guinean isolate. The resulting reads were assembled into a single contiguous sequence and annotated with reference to the complete mitochondrial genome sequence published for the filarial nematode *Brugia malayi*. We find that the Wb mt genome is 13,637 nucleotides in length and contains 36 genes that are typically found in metazoans. Encoding 2 ribosomal RNAs, 22 transfer RNAs, and 12 protein-coding genes, this genome is characterized by a high AT content (74.6%). In addition to using start codons identified previously in the mitochondrial protein-coding genes of other filarial nematodes, Wb mt DNA appears to be unique in its use of TGT as a start codon. Similarly, use of incomplete stop codons in mt protein-coding genes appears to be more common in Wb than in other human filarial parasites. The mt gene order for Wb is identical to that reported for O. volvulus, D. immitis, S digitata and *B. malayi* but is distinctly different from the other nematodes compared. In conclusion, the complete mt genome sequence reported here provides new genetic markers that may be used to monitor the progress of public health interventions aimed at control and elimination of this important human parasite. This data has facilitated preliminary exploration into Wb diversity and will be helpful for future studies aimed at population genetic and molecular epidemiology of LF.

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MITOCHONDRIAL CYTOCHOME OXIDASE I (COXI) SEQUENCE POLYMORPHISM REVEALS POPULATION GENETIC DIVERSITY OF *WUCHERERIA BANCROFTI* IN PAPUA NEW GUINEA

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Wuchereria bancrofti (Wb) is the primary causative agent of lymphatic filariasis (LF), a deforming and debilitating disease estimated to affect 120 million people in 83 countries. A global chemotherapeutic program to eliminate LF by mass drug administration has been introduced. This large-scale chemotherapeutic approach is likely to result in changes in the genetic structure of Wb populations. In this study, blood samples were collected from individuals from 4 villages in the Dreikikir district, East Sepik Province in Papua New Guinea (PNG). These villages represent high and moderate transmission areas (High: Peneng, Kilmangleng, Albulum; Moderate: Moilenge). Wb-positive samples were identified using a post-PCR LDR-FMA described previously. One positive sample from each of the four villages was used in this study, in which a portion of the cytochrome oxidase I gene (680 bp) was amplified, cloned, and sequenced to study

polymorphism and determine the extent of genetic heterogeneity. Among the resulting 38 sequences, 35 haplotypes (Haplotype diversity [Hd]= 0.98) and 99 polymorphic sites (101 mutations) were observed. Diverse populations of Wb were detected in both high (Peneng, Hd= 0.956; Kilmangleng, Hd= 0.9; Albulum, Hd= 0.923) and moderate (Moilenge, Hd= 0.957) transmission villages. Two haplotypes were shared between villages; one haplotype between individuals in Peneng and Kilmangleng, and one shared among individuals in Kilmangleng, Albulum and Moilenge. Additionally, one haplotype was represented twice in individuals from Kilmangleng. The present study suggests that genetically diverse Wb populations exist within and between the four PNG villages studied, as well as within the four individuals studied. A better understanding of the genetic structure of Wb populations may provide important insights into patterns of transmission, disease outcome, and anthelmintic drug resistance, and may thus inform the design and implementation of public health interventions aimed at eliminating LF.

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USING MICROFLUIDIC DEVICES FOR METABOLITE PROFILING OF *BRUGIA MALAYI* HOST-PATHOGEN INTERACTIONS

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Parasitic filarial nematodes such as those that cause lymphatic filariasis (*Brugia malayi, B. timori, Wuchereria bancrofti)* and river blindness (*Onchocerca volvulus*) put nearly 2 billion people at risk in some of the world's poorest countries. These parasites can survive innate and adaptive immune challenge for over a decade despite occupying immune-rich niches such as the skin and lymphatic vessels. A number of immunomodulatory effects have been attributed to filaria, including deficient antigen presentation, induction of regulatory cell populations, suppression of Th1- and Th2- associated cytokine production and altered effector cell recruitment. While some macromolecules with immunomodulatory properties have been isolated from filarial secretions, there has been little effort directed towards studying small molecules secreted by filarial parasites. Building upon our previous studies using LC/MS metabolomics to identify a serum biomarker set for onchocerciasis infection, we have designed and built a 'lab-on-a-chip' system to facilitate the general study of parasite/host interactions. These devices are simple to construct and allow for uni- and bi-directional signaling between immune cells and parasites, facilitating both phenotypic assessment as well as periodic sampling for metabolomic studies. Using these devices, we have studied the interaction between components of the human immune system (e.g., eosinophils, neutrophils) and *B. malayi* by LC/MS-based metabolomics. In this lecture, the molecular profiles of exposing immune cells to the parasite secretome, and vice versa, will be discussed.

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FROM GENE TO VACCINE FOR *BRUGIA MALAYI*: APPLYING IMMUNOINFORMATICS TOOLS TO NEGLECTED TROPICAL DISEASES

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Lymphatic filariasis (LF), which causes the well-known clinical manifestation of elephantiasis, afflicts over 120 million individuals worldwide with another 1.34 billion at risk of infection due to living in endemic regions. While current treatments are effective at lowering parasitemia within infected individuals, no treatment is available that prevents infections from occurring. To effectively prevent infection and to meet the goals of disease elimination set by the WHO, a vaccine would be very helpful. In this study, we used oligonucleotide microarrays to study changes in gene expression during the important transition of *Brugia malayi* from the infective stage in the mosquito vector (L3 mosquito) to the infective stage following entry into the mammalian host (L3 jird). Then, through use of the matrix algorithm EpiMatrix, we identified T cell epitopes in proteins that are upregulated during this transition. To identify possible vaccine candidates, we adopted the genome-to-vaccine design described by Arditto et al. (2010). This approach hinges upon two main principles: "(i) a minimal set of immunogens capable of inducing a robust and sustained immune response to a pathogen can be discovered using immunoinformatics, and (ii) administration of these immunogens, in a suitable delivery vehicle together with adjuvant, will result in protection from disease." This method of vaccine design delivers only what is needed to obtain protection, eliminating unnecessary material and the potential for adverse reactions. Out of 178 genes seen to be up-regulated, 23 were predicted to be highly immunogenic (e-value ≥50). Of these, 9 are predicted to be secreted, making them even better vaccine candidates. As the number of proteins that are being considered for vaccine development expands, rapid, inexpensive and accurate tools are in great demand. The approach described here may significantly accelerate the development of a vaccine for lymphatic filariasis.

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MOLECULAR DIAGNOSIS OF SCHISTOSOMIASIS FOR EPIDEMIOLOGICAL STUDIES

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Demonstration of parasite eggs in urine samples by microscopy is still the method of convenience in most epidemiological studies of schistosomiasis despite its numerous disadvantages including most especially low sensitivity. Recently an ITS-based real-time PCR for the detection of *Schistosoma* species DNA in both urine and faeces samples has been developed which shows a high sensitivity and specificity for schistosoma DNA amplification. This protocol was tested in the field in schistosomiasis endemic areas in Ghana using a total of 730 urine samples collected from school children in comparism with results of microscopy analysis. The samples analysed for the presence of schistosome eggs and DNA using microscopy and real-time PCR respectively. A high proportion of samples without detected eggs by microscopy were included to obtain a more precise estimation of the test sensitivity. Out of the total samples analysed, 8.9% (57) were found to contain schistosoma egg by microscopy detection where as PCR amplification revealed the presence of *Schistosoma* species DNA in 20.8% (152) of the samples. Taking into consideration true positives as microscopy positives and/or PCR positives, positive predictive value calculated was 100% for each school sampled an indication of correct diagnosis of *Schistosoma* positive sample. Of the 152 PCR positives, 59 (38.8%) had Ct values above 35 cycles majority (94.9%) of which were egg negatives. In 102 (15.1%) of the samples testing negative with microscopy, PCR detected DNA amplification with most of these samples exhibiting Ct values less than 35. A good correlation was observed for high intensity infections (egg counts of >50 eggs per 10ml urine), for which PCR samples tested positive for all with low Ct values (<30) indicating higher DNA loads. Results of this study show PCR to be significantly sensitive than microscopy for the detection of the presence of the parasite in the population and evaluating the intensity of infection, which is an important aspect of epidemiological studies. ITS-based multiplex real-time PCR can as such serve as a powerful tool in epidemiological surveys of schistosomiasis in providing more precise results than microscopy as well as eliminating many of the negative drawbacks associated with parasitological analysis.

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EOSINOPHIL CATIONIC PROTEIN AS A POTENTIAL PROGNOSTIC MARKER FOR INFECTION INTENSITY DETERMINATION IN A *SCHISTOSOMA*-ENDEMIC COMMUNITY IN GHANA

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There is increasing demand for more accurate and non-invasive methods in determining infection intensity in schistosomiasis. Recent studies have shown the urine filtration and Kato-katz techniques to grossly underestimate intensity of infection with *S. haematobium* and *S. mansoni* respectively. Accuracy in infection intensity determination by these methods improves only with increasing number of samples collected per participant. This however is tedious and time-consuming, hence increasing chances of experimental errors. This study sought to determine any association between levels of Eosinophil Cationic Protein (ECP) and infection intensity both in *S. haematobium* and *S. mansoni* single and co-infections.The study was conducted in Pakro, a peri-urban community in the Akuapem-South district of the Eastern Region of Ghana. A total of 254 participants: 124 males and 130 females; aged 6 to 96 years, were involved. Each participant provided up to 50ml urine samples and at least, 2g stool samples, which were processed using the filtration and Kato-katz techniques respectively, and examined by microscopy. Aliquots of urine from 73 participants, aged 6 to 40 years were analyzed for ECP levels using the Mesacup ECP-ELISA kit (MBL International). Thirty-nine were *S. haematobium* egg-positive, 2 were positive for both parasites, and 32 were egg negative. Of the 254 urine samples examined, 59 (23.23%) were positive for *S. haematobium*, 1 for *S. mansoni*, and 2 for both parasites. Also, ECP positively correlated with infection intensity by egg count ($p < 0.001$). Higher levels of infection intensity was observed among males ($p < 0.05$). Mean ECP levels were found to be significantly higher in *S. haematobium*-positive, than in *S. haematobium*-negative samples ($p < 0.001$); and almost twice as high for mixed, than for single infections. The ECP ELISA technique showed high sensitivity, but lower specificity. Further research is needed to improve specificity and ascertain ECP levels for mixed infections in schistosomiasis.

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HUMAN ANTIBODY RESPONSE TO THIOREDOXIN PEROXIDASE-1 AND TANDEM REPEAT PROTEINS AS IMMUNODIAGNOSTIC ANTIGEN CANDIDATES FOR *SCHISTOSOMA JAPONICUM* INFECTION

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Since its discovery in 1851, schistosomiasis has continued to be a public health problem in many tropical and subtropical countries and is far from being eradicated in spite of national control programs implemented in endemic areas. Schistosomiasis diagnosis plays a major role in evaluating the efficacy of such control programs. Improving therefore the diagnostic tools for surveillance and monitoring in areas which have reached elimination level will help hasten the possible elimination of this disease. In this study, we assessed the immunodiagnostic potential of thioredoxin peroxidase-1 (SjTPx-1) and 4 tandem repeat proteins (Sj1TR, Sj2TR, Sj4TR, Sj7TR) using human samples. This study therefore aims to develop ELISA through the use of these recombinant proteins. Cut-off values were calculated using 20 serum samples from healthy Japanese volunteers. Eighteen schistosomiasis-confirmed human samples were used to assess these antigens. Results showed that SjTPx-1 and Sj7TR both have 77.8% sensitivity which may be further improved by complementing these 2 antigens. Furthermore, these antigens were also tested against *Plasmodium falciparum*, *P. vivax* and *Entamoeba histolytica* positive sera and showed no cross-reaction between these parasitic infections. These results suggest the potential of these defined antigens for development of a more reliable diagnostic test for schistosomiasis.

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DEVELOPMENT OF A SEROLOGICAL DIAGNOSTIC ASSAY FOR *SCHISTOSOMA MANSONI* USING RECOMBINANT SM25

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The microsomal antigens (MAMA) of *Schostosoma mansoni* have been used as diagnostic antigens with great success for twenty years. Tests using MAMA are accepted as one of the most reliable assays for the accurate detection of low-burden infections, exposure, and identification of cases in endemic areas with a low rate of transmission. There are two diagnostic bands in MAMA, Sm25 and Sm29. Antibody reactivity with either of these proteins indicates a current or previous infection with *S. mansoni*. Because of the cost of adult worms and the complexities of preparing MAMA, it is critical to develop recombinant proteins of these diagnostic antigens. We purified, sequenced and cloned the integral membrane protein, Sm25. A hydrophilic stretch of 90 amino acids from Sm25 protein sequence was expressed in a baculovirus expression system with a GST tag (rSm25). In this study, we developed and evaluated an enzyme-linked immunoelectrotransfer blot (EITB) using rSm25 for laboratory identification of schistosomiasis. We analyzed a panel of 374 sera composed of 189 sera from parasitologically confirmed cases, 110 sera from persons with other parasitic infections and 75 from persons with no documented illnesses (normal sera). The optimized assay has a sensitivity of 91.2% and specificity of 97.2%. Our results suggest rSm25 EITB assay may be valuable in detecting *S. mansoni* infections and may make this assay more widely available.

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GLYCAN BASED DIAGNOSTIC ANTIGENS DISTINGUISH ACTIVE FROM FORMER INFECTIONS IN EXPERIMENTAL SCHISTOSOMIASIS MANSONI

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Like many other neglected tropical diseases, the control strategy for schistosomiasis consists of mass drug administration (MDA). However, current assessment of MDAs for schistosomiasis is limited because the available serologic diagnostic tools lack the capacity to distinguish current from former infections, even after successful chemotherapy. This limits the ability to rapidly monitor program impact or clearly indicate whether or not there is a need for repeat MDA. Because the world's current production of praziquantel, the only drug available for schistosomiasis MDA, is much less than the number of persons who need treatment, judicious use of drug is of utmost importance. A sensitive and specific diagnostic tool that can rapidly detect active infection is critically needed to evaluate the efficacy of control programs and to help determine the most cost effective approaches for schistosomiasis control. Antibody responses in schistosome-infected mammalian hosts are directed primarily against carbohydrate epitopes on schistosome worm surfaces and their secreted products. This suggests that carbohydrate antigens could be useful as sero-diagnostic tools for schistosomiasis. However, the evaluation of schistosome carbohydrate antigens has been limited by the challenges associated with generating large quantities of specific glycan structures for testing. In a previous study, use of glycan arrays demonstrated that epitopes terminating with beta (1, 2)-xylose (core-xylose) and alpha (1, 3)-fucose (core-fucose) were strongly recognized by serum of infected mice, rhesus monkeys, and humans. We have now generated these glycan

epitopes from naturally occurring plant and animal products, conjugated them to specific carrier proteins, and tested their utility as sero-diagnostic tools by ELISA. In longitudinal studies on rhesus macaques, they are recognized during active infection but the antibody response disappears after clearance of schistosomes. Preliminary data suggest a similar temporal recognition pattern in humans with active schistosomiasis and following treatment.

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THE DEVELOPMENT OF A NOVEL MULTI-CHANNELED SEROLOGICAL ASSAY FOR THE DETECTION OF IGG4 ANTIBODY LEVELS VERSUS MORE TRADITIONAL METHODS TO ESTIMATE THE BURDEN OF DISEASE OF URINARY SCHISTOSOMIASIS AND LYMPHATIC FILARIASIS IN A POPULATION ON THE COAST OF KENYA

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Coinfection with multiple parasites is common in the developing world. To better understand the impact of polyparasitism in a population in coastal Kenya, we developed a novel, serum sparing, multi-channel fluorescent serological assay that simultaneously measures serum or plasma IgG4 against *Brugia malayi* antigen (BMA) and *S. haematobium* soluble worm antigen (SWAP). IgG4 was chosen for diagnosis because it indicates active or recent infection and represents the most specific IgG isotype response against BMA or SWAP. Our new IgG4 bead assay was compared to ELISAs for anti-SWAP and anti-BMA IgG4 and to standard parasitologic diagnoses for lymphatic filariasis (ICT antigen detection card) and urinary schistosomiasis (urine filtration). Bead assay cut-off values were determined using control sera from a different area of Kenya that is endemic for multiple parasites but not lymphatic filariasis or urinary schistosomiasis, i.e., positive samples had fluorescence 3 SD above mean values for control sera. Compared with IgG4 ELISA as a gold-standard diagnostic, the novel multi-channeled assay had a sensitivity of 100% and a specificity of 58% with regard to BMA antibody response and a sensitivity of 98% and specificity of 57% with regard to SWAP. However, the IgG4 fluorescent bead assay was more sensitive and specific when compared to the filarial card or urine filtration. Overall, IgG4 by multiplex assay also correlated well with infection-associated morbidity at the population level. Measuring antibody levels by fluorescent bead microassay may not only increase the sensitivity of testing for these diseases, but could provide more useful epidemiological information than standard parasitologic tests.

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APPLICATION OF GOOGLE EARTH AND WEB-BASED GEOGRAPHICAL INFORMATION SYSTEMS (WEB GIS) ON THE REAL-TIME MONITORING PLATFORM FOR SCHISTOSOMIASIS TRANSMISSION

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The application of geographic information systems (GIS) on epidemiological study of schistosomiasis are rapidly growing since the late 1980s in China. However, there are still many obstacles for furthermore application and development. The technology of Internet and virtual globe technologies Google Earth enable scientists, professionals in disease control or decision makers early access to the GIS servers to share their data and findings in a visually attractive without the need for highly sophisticated GIS or much technical assistance. This study elaborated the basic framework and structure in fast risk assessment of schistosomiasis transmission based on Web-based geographical information system (Web GIS), Google Earth, which initially designed with functions in information collection, search and evaluation and analysis of risk factors, dynamic prediction and dynamic early-warning and functions of guidance and management in this system. The design of this system provided evidence based and real-time information to understand the endemic status of schistosomiasis transmission, release real-time information and properly take quick response to transmission foci. The evaluation from running system showed that the combination of two venues, e.g. Google Earth and Web GIS, will have tremendous potential to strengthen overall monitoring and evaluation capacity and facilitate the support system to strengthern the capacity of schistosomiasis control.

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ASSESSING THE POTENTIAL OF *MACROBRACHIUM VOLLENHOVENII,* A LARGE FRESHWATER PRAWN, TO CONTROL SCHISTOSOMIASIS TRANSMISSION IN THE SENEGAL RIVER BASIN

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Schistosomiasis is one of the most common parasitic diseases of humans, and Senegal contains some of the highest transmission sites for schistosomiasis in the world. In Senegal, schistosomiasis outbreaks are thought to have been exacerbated by the building of the Diama Dam in 1986, since the dam increased freshwater habitat for aquatic snail intermediate hosts of schistosome parasites. Controlling snail populations, therefore, is a logical step in the control of schistosomiasis transmission. However, the drug praziquantel has become the predominant tool of schistosomiasis control programs, and biological control of snails has received little attention in comparison. One of the most promising candidates for biological control of schistosome-hosting snails in West Africa is the native prawn, *Macrobrachium vollenhovenii*. We hypothesize that prawns were a common snail predator in the Senegal River, but the Diama Dam posed a barrier to prawn reproduction, and hence, prawn populations have declined, contributing to the emergence of schistosomiasis. In this study, we assess the current population dynamics of free-ranging *Macrobrachium* spp. prawns in the Senegal River Basin, as a first step towards assessing the potential of prawn re-introduction as a snail control strategy. We examine 11 sites, surveyed bimonthly over a period of one year, and record water quality, relative snail abundance, and prawn distributions. We find that freshwater snail abundances are high and seasonally variable, and schistosome intermediate host species predominate at most sites. Prawn abundances are low at all sites but are highest at downstream sites near the river mouth and below the dam. Water quality parameters pH, temperature, nitrate, nitrite, phosphate, alkalinity, and Ca^{++} hardness are within the ranges reported acceptable for *Macrobrachium* growth and survival at most sites. Anecdotally, local fishermen report that native prawns had been a viable fishery. However, catches have declined dramatically in the river basin over the last few decades. Our results suggest that aquaculture of *M. vollenhovenii* prawns warrants serious consideration as a biological control strategy for schistosomiasis. In addition to their potential as snail predators, *M.* spp. prawns are a valuable global commodity, and thus, prawn aquaculture may be a promising tool for both disease control and poverty alleviation.

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POPULATION GENETICS AND EPIDEMIOLOGY OF *SCHISTOSOMA MANSONI* IN SYMPATRIC HUMANS AND NON-HUMAN PRIMATES IN THE GOMBE ECOSYSTEM TANZANIA

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Increased contacts between humans and wildlife around Gombe National Park in western Tanzania have raised the risk of disease sharing amongst them. Although both humans and non-human primates in the area have schistosomiasis, it is not known whether strains of their schistosomes are epidemiologically and genetically distinct. The genetic ecology of Biomphalaria snails, the intermediate hosts for schistosomes in the area is also not well known. This study investigated the infection patterns of schistosomiasis in humans and non-human primates in Gombe and surrounding villages of Mwamgongo, Bugamba, Kiziba and Mtanga. It also examined if there is genetic mixing of snails between village streams and whether they can spread parasites between them. Snails were collected using a scoop and exposed to light for shedding schistosome larvae. Representative snails were dissected and preserved in RNA-later for molecular analysis. Faecal samples obtained from 16 vervets and 110 baboons were examined for parasites using formol-ethyl acetate technique. Stool samples from 55 people in Gombe National Park and about 80 people from each village were examined for parasites using the Kato-Katz technique. Additional stool obtained from 41 people and 9 baboons was analyzed using molecular methods to characterize the genotypes of their schistosome parasites. Overall, the prevalence of *Schistosoma mansoni* in humans was 45.05% and 11.24% in baboons, with no such infection in vervets. Other parasites diagnosed in humans included Trichuris trichiura (1.49%), *Ascaris lumbricoides* (0.99%), and Taenia sp. (0.25%). Molecular analysis of human and baboon faecal eggs based on sequencing of the small subunit rRNA region confirmed the parasites to be *S. mansoni*. More variable genetic markers will be screened to establish the genetic relationships between human and baboon strains of schistosomes. Based on shedding, none of the snails from Gombe and Bugamba had schistosome larvae while 22.64% from Mwamgongo, 22.58% in Kiziba and 16.62% from Kigoma town were infected. PCR-based tests will be conducted to determine whether snails that did not shed parasites could be infected. The implication of these infections to human and animal health in the Gombe area will be explored. Information obtained will help to understand the epidemiology of schistosomiasis and facilitate control programmes for the disease both in humans and wild animals in the area.

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SCHISTOSOMIASIS IN CATTLE HERD BOYS: POSSIBLE IMPACT ON SCHOOL-BASED CONTROL STRATEGIES IN THE DANGME EAST DISTRICT OF GHANA

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The fundamental strategy of controlling the morbidity of schistosomiasis is through mass administration of praziquantel, in school-aged children. This strategy may not achieve the desired goals if it is limited to a school-based mass treatment approach that excludes children who are not enrolled in school. The out of school children could act as sources of re-infection. We evaluated the possibility of cattle herd boys as reservoirs of *Schistosoma* species that could lead to re-infection in treated communities. The study was undertaken in the Dangme East District of Ghana where in a previous cross sectional study in 2006, 1,030 school children (aged 6-17 years) screened indicated a prevalence of 8.4% (86/1030) and 0.3% (3/1030) with the intensity of infection ranging between 4-493 eggs per 10ml and 1 to 6 epg in *S. haematobium* and *S. mansoni* respectively. We conducted a four-month (September-December 2010) study on 17 cattle herds boys aged 8-18 years. This involved stool and urine examination by kato-katz and urine-filtration techniques, and confirmed with real time PCR. We used GPS to map the trail of the cattle herdsmen and water sites frequented during their activities. An infection prevalence of 35.3% (6/17) and 11.8% (2/17) *S. haematobium* and *S. mansoni*, with egg counts between 2 to 212 per 10ml and 4 to 6epg respectively were observed. The map of the trail used by the cattle herd boys during their activities indicated that the grazing happens in close proximity to the communities and schools with the water sites (for the cattle) utilized by the herd boys, school children and the communities. The infection level in the cattle herd boys and the proximity of schools to the grazing trail and water sites could influence infection and re-infection among school children in the communities studied. These preliminary findings could be followed up using a larger population of cattle herdsmen over a larger area.

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MULTI-COMPONENT INTEGRATED CONTROL FOR THE ELIMINATION OF SCHISTOSOMIASIS FROM CHINA: STUDY DESIGN AND BASELINE RESULTS

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Zoonotic schistosomiasis japonica is a major health risk for more than 40 million Chinese with a million people and several hundred thousand livestock infected. Major endemic foci occur in the lake (Dongting and Poyang) and marshlands along the Yangtze River basin; elimination of transmission has proved difficult. For the past 50 years the Chinese government has made great strides in controlling a disease regarded as a public-health priority, but this is predicted to change as a consequence of the completion of the Three Gorges Dam (TGD), which crosses the Yangtze River. The environmental and ecological impacts will result in exponential expansion of the habitat for the intermediate snail host *Oncomelania hupensis*, increasing the risk of human and bovine infection, resulting in potentially severe consequences for control. We have shown that bovine infections are responsible for the persistence of human schistosomiasis transmission in China. Schistosomes debilitate infected domestic livestock which are used for food and as work animals, consequently adding to the economic burden and suffering of endemic communities. Transmission reduction is a key step in eliminating schistosomiasis, but current praziquantel-based control programs are unable to achieve this due to the inability of praziquantel to prevent re-infection. We propose that a multi-component integrated approach (incorporating praziquantel chemotherapy, molluscicding and bovine vaccination) targeting the various transmission pathways is required for sustainable control and elimination in response to the changing environment as a result of the TGD. In 2010 we commenced a 5-year intervention trial to determine the impact of multi-component integrated control on schistosome transmission. Here we present the study design and baseline results.

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SCHISTOSOMIASIS IN MOTHERS AND INFANTS (SIMI) FROM UGANDA: KEY FINDINGS FROM A CLOSED COHORT LONGITUDINAL STUDY ADMINISTERING PREVENTIVE CHEMOTHERAPY

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In certain parts of Uganda, the transmission of intestinal schistosomiasis can be particularly intense such that very young children (< 5 years of age) can be evidently infected with *Schistosoma mansoni*. Indeed, in many lakeshore communities, young children come into daily contact with schistosome cercariae owing to the domestic use of freshly drawn lake water by their respective mothers; the prevalence of egg-patent infections can be in excess of 50% in children under 3 years of age. From a public health perspective, treatment of these younger children with praziquantel is being explored as international guidelines need to be revised if better access to medications for this group is required. Therefore convincing evidence from on-the-ground studies is essential to demonstrate a clear need and likely benefit for revision of international policies for control. The schistosomiasis in mothers and infants (SIMI) project, funded by The Wellcome Trust, has now completed 18-months of close epidemiological surveillance and supervision of praziquantel treatment of over 1,500 young children in 6 shoreline villages of Lakes Albert and Victoria, Uganda. Using a combination of new diagnostic tools, epidemiological monitoring and clinical markers the key findings of this project will be presented. Foremost, hurdles and solutions will be discussed but, above all, the pressing need for roll-out of control to this ageclass will be strongly advocated as, not doing so, is increasingly untenable.

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APPLICATION OF MICROSATELLITE GENOTYPING TO CERCARIAE IN THE INVESTIGATION OF URBAN SCHISTOSOMIASIS

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Historically, schistosomiasis has been described as a rural disease, however, urban transmission is more and more commonly observed in cities of Brazil. Our goal was to determine the utility of cercariae shed from collected snails for assessing genetic relationships between urban populations of parasites. In an ongoing malacologic study of all major collections of water in the city of Salvador (total 158), 7 were positive for infected snails. Cercarial DNA from 5 sites was extracted and quantified by qPCR. Jost's D differentiation index was determined based on genotypes from 14 microsatellite markers. Worm and cercarial DNA from laboratory strains maintained at Case Western Reserve University and at Oswaldo Cruz Foundation, respectively, were genotyped for comparison and as positive controls. Eggs collected from 9 infected children in the neighborhood of São Bartolomeu were also genotyped. The total number of alleles observed for all markers and samples was 120 (range 44 - 91). The average effective allele number (Ae) was similar across all cercarial samples (mean 2.31), but largest in stool eggs (Ae = 3.96). A pairwise comparison of the Jost's D values of all cercarial collections showed a high degree of differentiation between them (mean 0.411), statistically no different than comparing field collected cercariae and controls (mean 0.395). Comparing the cercariae collected from snails on the main river of São Bartolomeu to infected children gave a Jost's D value of 0.505, indicating very different populations. Only two collections suggested potential gene flow between them, the laboratory strain Feira de Santana and cercariae from a small lake near São Bartolomeu (Dique do Cabrito' mean Jost D = 0.017). This, however, is spurious since they are reproductively isolated from each other. Therefore, there was no correlation between geographic location and genetic similarity. While examination of snails for infection may be an important tool for evaluation of transmission, it may not be useful to assess parasite population structure and dynamics in the human host.

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REGULATORY T CELLS IN HUMAN SCHISTOSOMIASIS BEFORE AND AFTER TREATMENT WITH PRAZIQUANTEL

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Human schistosomiasis, one of the most common parasitic infections worldwide, is associated with down-regulation of host immune responses. It has been suggested that regulatory Tcells are induced by schistosomes to allow their long term survival within an immunocompetent host. Here, we study the frequency of CD4+CD25hiFoxp3+ cells in peripheral blood of subjects with and without *Schistosoma haematobium* infection and assess their suppressory activity by comparing total and CD4+CD25hi -depleted PBMCs. Proliferation and cytokine production was measured in response to schistosome egg antigens (SEA) and the vaccine-antigen BCG. Infected children were treated with praziquantel and regulatory T cells were assessed 6 week post treatment. Higher numbers of peripheral blood CD4+CD25hiFoxp3+ Treg cells were found in *S. haematobium* infected children compared to non-infected control subjects. Six weeks after treatment, proliferative responses to antigens tended to increase while there was a significant enhancement of Th2 cytokines in response to schistosome antigens and Th1 cytokines in response to BCG. The number of regulatory T cells decreased by 50%, and their suppressive activity on proliferation as well as on IL-5 to SEA and TNFα to BCG, diminished after treatment. Taken together these data suggest that *S. haematobium* infection is associated with upregulation of regulatory T cells and downregulation of certain antigen specific responses.

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AGE-STRATIFIED SERUM CYTOKINE PROFILE (IL-6, IL-10, TNF-A) IN KENYAN CHILDREN WITH EARLY *SCHISTOSOMA HAEMATOBIUM* INFECTION

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In a study of children with polyparasitic infections in a *Schistosoma haematobium* (Sh) endemic area, we examined the hypothesis that infection-associated inflammation precedes detection of *Sh* infection by standard urine filtration. Children 5-18 yr old were surveyed in August - October 2009, and tested for *Plasmodium falciparum* by ICT card and for *Sh* both by urine filtration and anti-SWAP detection. IgG4 anti-SWAP positive children (n=221) were compared to anti-SWAP-negative children (n=62) for levels of pro-inflammatory cytokines IL-6, TNF-α, and down-regulatory IL-10. In the α-SWAP positive children, regardless of age, there were higher serum IL-6 levels compared to α-SWAP negative children, with

the greatest difference seen at 11-13 yr (mean 5912 ng/ml). Increased serum IL-6 correlated with parasitic infection, anemia, and acute and chronic malnutrition. IL-10 levels peaked at 9-11 yr in the α-SWAP positive group (mean 430 ng/ml) and were inversely correlated with IL-6 levels. Children in the α-SWAP positive group and infected with hookworms and *P. falciparum* had significantly increased serum levels of IL-10 (*P*=0.045 and *P*= 0.015). Elevation of TNF-α in the α-SWAP group was also associated with malaria infection in 7-9 yr olds (*P*=0.009*).* Our results show a marked difference in the cytokine profile among α-SWAP positive *vs.* α-SWAP negative children, with an early inflammatory response in α-SWAP positive young children (5-7 yr old), measurable by increased IL-6 and low IL-10, before eggs are detected in urine. Schistosomiasis-malaria co-infection strongly correlated with higher pro-inflammatory cytokines in serum, suggesting an important morbidity-related interaction between these parasite species in children.

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SPATIAL ANALYSIS OF DETERMINANTS OF DENGUE TRANSMISSION WITHIN A PROSPECTIVE COHORT STUDY IN VENEZUELA

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Control of dengue and of its mosquito vector has proven challenging in settings of uncontrolled urban growth and unreliable water supply. The ability to identify high-risk areas of dengue transmission can be used to target surveillance and control measures to those locations in a cost-effective manner, particularly in countries where resources are scarce. Mapping technology and spatial analysis of epidemiological data will be used to draw risk-maps and identify key factors that determine clusters of high dengue transmission and the spatial spread of dengue within a prospective cohort in Maracay, an endemic city of Venezuela. 2000 individuals aged 5-30 years have been enrolled between August-December 2010 into a cohort study. Geolocation of households, water bodies and other environmental factors as well as epidemiological data comprising demographic, socioeconomic, clinical, serological and hematological data were collected at baseline. Annual cross-sectional surveys will determine seroconversion and collect further epidemiological information. Active and passive surveillance is performed to identify dengue cases. Collected data will be imported into geographic information systems software for spatial statistical analysis (regression models) at household level. Risk maps of dengue occurrence measured as confirmed cases by RT-PCR and/or serology both overall and stratified by serotype will be presented. The effect of serotype-specific transmission will be explored. Preliminary results and implications for dengue control will be discussed.

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VARIATION IN DENGUE VIRUS PLAQUE REDUCTION NEUTRALIZATION TESTING: SYSTEMATIC REVIEW AND POOLED ANALYSIS

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The plaque reduction neutralization test (PRNT) is the gold standard for quantifying antibody responses against dengue virus (DENV). Despite the importance of comparable results in diagnostics and vaccine development, the effects of differing laboratory techniques, particularly the use of different viral strains within a dengue serotype, have not been well-characterized. This systematic review and pooled analysis aims to characterize differences in laboratory methods between articles reporting PRNT titers and quantify the effect of these differences on measured PRNT titers. We identified 32 articles reporting 4,411 titers from 605 individuals enrolled in vaccine trials (8 articles), serological surveys (3 articles) or observational studies (23 articles). These articles reported the use of 4 different neutralization end points, 3 different cell lines, 12 different virus concentrations and 51 different virus strains (9 for DENV1, 17 for DENV2, 17 for DENV3 and 8 for DENV4). Pooled analysis showed that the strain used in PRNT assays had a substantial effect on the measured titer, and accounted for 5% (90% credible interval: 1%, 11%) of inter-observation variation after adjusting for other factors. Differences between articles (in part a proxy for inter-laboratory differences) accounted for 37% (90% credible interval: 27%, 61%) of inter-observation variance after adjusting for other factors. These results call into question the comparability of dengue PRNT titers reported from different laboratories. These results highlight the importance of standardizing PRNT methods in order to permit inter-laboratory comparisons and reduce variability between study results.

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DENGUE VIRUS E PROTEIN DOMAIN III-REACTIVE ANTIBODIES IN POLYCLONAL IMMUNE SERA AND THEIR ROLE IN PROTECTION OR ENHANCEMENT *IN VIVO*

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The four dengue virus serotypes (DENV1-4) are responsible for the most prevalent arboviral disease in humans. The DENV virion surface is composed of 180 copies of envelope (E) protein, which is comprised of three domains (EDI, II and III). E is the main target of neutralizing antibody, and studies with mouse monoclonal antibodies indicate that antibodies that bind to EDIII can strongly neutralize DENV. Few studies have explored the properties of antibody subpopulations in polyclonal immune sera responsible for DENV neutralization and enhancement. Recent studies with human sera indicate that anti-EDIII antibodies contribute little to binding or neutralizing potency of human immune sera *in vitro.* The goal of this study was to assess the role of anti-EDIII antibodies in immune sera in neutralizing or enhancing DENV *in vivo*. We show that mouse dengue-immune sera have more EDIII-reactive, neutralizing antibodies than human immune serum. Using a depletion strategy to remove EDIII-specific antibodies from polyvalent serum, we demonstrate that anti-EDIII antibodies in DENV-immune human serum are not required for reduction of viral load after infection with a homologous serotype in our AG129 dengue mouse model, consistent with *in vitro* neutralization data. Depletion of anti-EDIII antibodies in mouse sera led to a significant reduction in neutralization potency. However, mice were protected by increasing the quantity of EDIII antibody-depleted mouse serum, indicating that the mice develop both EDIII-reactive and -non-reactive neutralizing antibodies. We also used our mouse model to evaluate whether EDIII-specific antibodies contribute to virus and disease enhancement *in vivo*. Depletion of anti-EDIII antibodies from mouse serum led to an increase in disease enhancement, indicating that anti-EDIII antibodies suppressed the ability of other antibodies in polyvalent immune sera to enhance infection. However, administration of larger amounts (greater neutralizing titers) of EDIII-depleted mouse serum was not enhancing. Finally, we conclude that neutralizing titer, measured in a flow cytometry-based assay with human U937-DC-SIGN cells, is a strong predictor of viral load and disease outcome *in vivo*, and serves as a better indicator than peak enhancement titer, as measured in K562 cells. This data supports the hypothesis that neutralization titer can serve as an important immune correlate of dengue vaccine-derived protection.

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THE IMPORTANCE OF PLATELET-DERIVED MICROVESICLES IN DENGUE PATHOGENESIS

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Platelets, the second most common cell-type in the circulation, are important for homeostasis of the body's physiology. They possess a unique feature- the ability to respond instantaneously to subtle changes in the surroundings and become activated. Platelet activation results not only in secreting tons of releasates but also unleashing platelet-derived microvesicles (PMVs) by shedding subsections of its membrane, a process similar to that of apoptosis. PMVs are the most frequently detected form of microparticle found in the circulation of human subjects. Cumulating evidence suggests that PMVs have significant pathophysiologic effects including the orchestration of inflammatory conditions. In addition to its role as a marker for cell damage, circulating cell-derived microvesicles are being recognized for their roles as signaling elements in cell-cell communication and as transport vesicles for proteins, nucleic acids and receptors in certain diseases and infections. However, the status of PMPs in acute dengue virus infected patients remains largely unexplored. PMVs were isolated from plasma of dengue patients by differential centrifugation. Viral RNA was quantified by qRT-PCR, viral isolation was done by co-culture with Vero cells, and proteomic profiling was performed in the isolated PMVs. In addition, some of the plasma proteins observed in proteomic were verified by ELISA. Results revealed that i) the levels of dengue viral RNA were significantly higher in the PMVs than in the platelet and serum fractions; ii) infectious virus could be recovered from the isolated PMVs, iii) although proteins with extracellular functions, chaperone and transport, and blood coagulation were dominant in isolated PMVs, several unique proteins were noticed, such as lactadherin and vitamin-D-binding protein; and iv) significantly lower lactadherin was observed in the plasma of dengue patients. These results indicated that dengue virus could be disseminated via PMVs in dengue patients and that the proteins associated with PMVs may have a protective role since lactadherin has been shown to have an enhancement activity in the clearance of PMVs by phagocytic cells. The information could be a critical step to further understand the complicated pathogenesis of dengue disease.

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RISK FACTORS FOR DENGUE SHOCK SYNDROME: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Several risk factors are reportedly associated with dengue shock syndrome (DSS), but the results from these reports are highly inconclusive. In order to estimate overall association of risk factors and DSS over dengue hemorrhagic fever (DHF), we systematically reviewed and performed a meta-analysis of relevant studies in both DSS and DHF patients. PubMed, EMBASE, Scopus, Google Scholar, Dengue Bulletin, Cochrane Library, Virtual Health Library, Cochrane Library, and manual search of reference lists of articles published before September 2010 were used to retrieve relevant studies. Two reviewers independently selected articles and extracted data on study characteristics and data regarding the association between factors and DSS over DHF in the form of 2x2 tables. A meta-analysis using fixed-effects or random-effects models to pooled odds ratios (OR) or difference in mean with corresponding 95% confidence intervals were calculated only if more than one study had investigated particular factor. We found 173 articles that met our eligibility criteria. Our meta-analysis showed that younger age, female, vomiting, jaundice, abdominal pain, hepatomegaly, gastrointestinal bleeding, hematemesis, ascites, pleural effusion, gallbladder wall swelling, DEN-2, thrombocytopenia, leukopenia, hematocrit increase over 20%, elevated ALT, prolonged APTT, prolonged PT were risk factors for DSS, whereas normal nutrition, DEN-1, DEN-4 were protective factors against the disease.

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CROSS-NEUTRALIZING ANTIBODY RESPONSES AGAINST CIRCULATING DENV FIELD ISOLATES AFTER HUMAN VACCINATION WITH A TETRAVALENT DENGUE VACCINE

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The potential of a vaccine to prevent natural infections may depend on the capacity of vaccine-induced antibodies to neutralize currently circulating strains. A tetravalent dengue vaccine (TDV) based on 4 recombinant, live, attenuated viruses (CYD1-4) has been developed at Sanofi Pasteur and is in clinical phase III evaluation. Serum neutralizing activity after vaccination is routinely evaluated against the vaccine parental, wild-type DENV strains: DENV-1/PUO-359, DENV-2/PUO-218, DENV-3/PaH881/88, and DENV-4/1228, all isolated between 1978 and 1988. We previously demonstrated that a pool of sera generated during preclinical evaluation of the vaccine in rhesus monkeys broadly neutralized contemporary DENV lineages of diverse geographical origin and genotype. This work presents data obtained in a similar evaluation, conducted with sera from volunteers currently enrolled in a phase II immunogenicity and safety clinical study in Singapore and vaccinated with TDV. Pools of serum were assembled from subjects according to the patient age group (adult / adolescent / children), the individual's flavivirus immune status before vaccination (positive / negative), and the post vaccination level of the neutralizing antibody response against the vaccine parental strains. For each serotype, 6 DENV strains were tested: two prototype strains (parent vaccine strains and WHO strains DENV-1/West Pac 74, DENV-2/S16803, DENV-3/CH53489 and DENV-4/TVP360), and 4 field isolates of different genotype and geographic origin, including 2 Latin American strains and 2 Asian strains. This data will be discussed with regards to their implications for human vaccination.

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TRAVEL-ASSOCIATED CASES OF DENGUE REPORTED TO THE CENTERS FOR DISEASE CONTROL AND PREVENTION 2006 THROUGH 2010

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Dengue is the leading cause of systemic febrile illness in travelers returning from dengue endemic areas of the Caribbean, Latin America, and Asia. We describe Travel-associated dengue cases occurring among persons residing in the 50 US states and the District of Columbia (DC) and illness onset during 2006 through 2010. Dengue case data reported to the Centers for Disease Control and Prevention's (CDC) national arbovirus surveillance system (ArboNET) and case data from patients with specimens submitted to the CDC Dengue Branch (DB) but not reported to ArboNET were analyzed. For ArboNET cases, laboratory result interpretations and travel classifications were determined by the reporting jurisdiction. For DB cases, laboratory-confirmed cases were patients with DENV RNA detected by real-time PCR. Laboratory-probable cases were patients with anti-DENV IgM antibodies detected by ELISA in a single convalescent specimen. Travel-associated illness was defined as dengue-like illness in a resident of the 50 United States and DC traveling abroad within 14 days of illness

onset. Between 2006 and 2010, 1,315 laboratory-confirmed or probable Travel-associated cases were reported (annual average = 263; p value for trend = 0.3); mean age was 40 years and 50% were male. Of those with clinical information, 89% (1,023), 5% (53), 3% (30), and 4% (44) were classified as dengue fever (DF), DF with hemorrhage, dengue hemorrhagic fever or shock syndrome or not classified, respectively. The average annual number of hospitalizations was 110 (p value for trend = 0.8) and deaths was 1 (p value for trend = 0.3). Over 54% of cases were reported from Florida, New York, Texas and Minnesota; > 45% of cases were attributed to travel to the Dominican Republic, Puerto Rico, India, Mexico, and Haiti. In conclusion, travel to dengue endemic areas continues to pose a risk to US travelers. Persons traveling to these areas should seek pre-travel consultation, minimize mosquito exposure while traveling, and seek medical attention if fever develops during travel or after return.

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THE ROLE OF ANTIBODIES IN DENGUE VIRUS PATHOGENESIS: UNDERSTANDING PROTECTION VERSUS ENHANCEMENT

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Primary infection with one Dengue virus (DENV) serotype generally confers lifelong homotypic immunity but only short-term heterotypic immunity to the other three serotypes. Secondary infection with a heterologous serotype typically results in severe Dengue Hemorrhagic Fever (DHF). Studies support a role for pre-existing antibody (Ab) to DENV in DHF pathogenesis through Antibody Dependent Enhancement (ADE), in which Abs induced by the initial infection enhance virus infectivity rather than neutralize leading to increased viral uptake into cells. Evidence supports a role for Fc-FcR interactions on non-neutralizing anti-DENV Abs in DENV pathogenesis. Elucidating the role of DENV-specific ADE in increased DENV infectivity, virus replication and viremia is the basis for this research. We hypothesize that non-neutralizing DENV Abs are responsible for increasing infectivity of host cells with heterologous DENV, and the increased viremia results in severe pathology. Memory B cells from convalescent DENV-infected patients were used to generate libraries of anti-DENV human monoclonal antibodies (HMAb) by molecular cloning. HMAbs were characterized based on DENV serotype specificity, cross-reactivity, antigenic binding sites, and neutralizing and/or enhancing ability *in vitro*. HMAbs with potent neutralizing activity against DENV-1 demonstrated decreased enhancement activity at higher concentrations, consistent with their presumed ability to block viral entry at full Ab site occupancy, whereas non-neutralizing Abs showed a positive correlation between enhancement and concentration. Thus both neutralizing and non-neutralizing HMAbs were able to enhance DENV infection *in vitro*; however, the degree of enhancement appears to be dependent on the concentration of the individual HMAbs. These HMAbs provide insight into the human immune response to DENV, which can be used to assess the role of Abs in DENV pathogenesis, specifically their ability to regulate the immune response to DENV, and may help determine Ab characteristics associated with protection versus enhancement of disease.

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HUMAN MOVEMENT DETERMINES RISK OF INFECTION WITH DENGUE VIRUS

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Knowledge of human mobility and how it influences pathogen transmission remains limited, especially at fine scales. We studied the importance of individual human movements, measured in terms of exposure to pathogen, for predicting risk of infection with dengue virus (DENV) over two transmission seasons dominated by DENV-4 in Iquitos, Peru. We used a survey to identify locations visited recently by 48 febrile individuals (23 DENV+, 25 DENV- controls) and sampled for evidence of acute dengue infection (RT-PCR, IgM ELISA) among contacts residing in those locations. Overall, we identified 97 total acute (PCR+ or IgM seroconversion) infections and 77 recent (elevated acute IgM) infections in 166 households (mean 3 contact houses per index case), 31% of which occurred >100m from the home. These data and serotype-specific plaque reduction neutralization test results from a prospective longitudinal cohort were then used to parameterize risk and attack rate models. Based on a simple theoretical model, we estimated exposure as a composite index of the number of recently visited locations with concurrent acute DENV infections, and the number of acute infections and susceptible hosts per location. We show that risk of infection with DENV is overwhelmingly driven by variation in exposure ($P<0.001$) and herd immunity ($P<0.05$). Attack rates in the activity spaces of DENV+ cases were markedly higher than DENV- controls (17% [95% CI:12-21%] vs 6% [95% CI:3-8%]), with no difference in household incidence between the home and contact sites of DENV+ clusters (16.9%). We conclude that human mobility is central to the transmission of this virus and for predicting who and what locations are at greatest risk for infection with DENV during an outbreak. We discuss the implications of our results for the design of dengue control and surveillance programs and argue our findings and methods are not specific to DENV and are relevant to understanding other infectious diseases.

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ECONOMIC COST OF DENGUE IN MALAYSIA: MERGING MULTIPLE DATA SOURCES

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While incidence of most infectious diseases has been declining worldwide, dengue cases are increasing. In 2010, the mainland United States experienced dengue transmission for the first time in decades. Vaccines and new mosquito control technologies are being tested, but their implementation will require additional resources. Information about economic burden is needed for setting priorities, but accurate estimation is difficult due to incomplete data. We are overcoming this limitation by literature review, engaging experts and data from both the health and surveillance systems, and using a Delphi process. The annual economic cost of a disease (e.g. dengue in 2009) can be calculated as the number of cases per year times the cost per case. While dengue is a reportable illness in Malaysia (e.g., 41,454 cases reported in 2009), the surveillance system is passive. To address possible underreporting of cases, we first obtained estimates of expansion factors (the number by which reported

cases need to be multiplied to obtain the true number) from previous studies in the literature and Malaysia's work permit system, FOMEMA. Private hospital laboratories found about 25,000 dengue-positive cases. Finally, clinicians estimated that about 50%-60% of dengue cases were treated in the ambulatory sector. Altogether, our first round expansion factors from 10 experts were 1.2 for reported hospitalized cases, 34.7 for reported ambulatory cases, and 2.3 overall, corresponding to 96,000 dengue cases per year. To estimate unit costs of dengue cases, we combined a publication from the University of Malaya, data from special studies, national health accounts, and inflation adjustments. Altogether, the 2009 annual cost of dengue illnesses was about US$ 36 million (US $1.20/capita). Of this, 41% was direct costs and 59% indirect costs; 66% of costs occurred in public sector cases and 34% in private sector cases. This study suggests that implementing a technology which would control dengue efficiently would be economically valuable.

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INTEGRATING HUMAN AND VECTOR MOVEMENT DATA INTO DENGUE VIRUS TRANSMISSION NETWORKS

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Human movement patterns and social structure play an important role in modulating human-vector contact rates, affecting transmission dynamics, and the spread and persistence of vector-borne pathogens. For dengue virus (DENV), limited dispersal range of its day-biting vector, *Aedes aegypti*, points to movement of viremic humans as a plausible explanation for the rapid spread of infection across urban environments. We used field data from spatially-explicit semi-structured interviews (SSI) and GPS data-loggers to derive contact networks of individual humans for DENV transmission in Iquitos, Peru. We obtained movement data for 1,200 participants and expressed their contact network as an undirected bipartite graph representing the locations participants had in common as a consequence of their routine movements. Vector dispersal was explicitly accounted for by linking locations within the hypothesized home range of the vector. Different measures of network topology were estimated for the full contact network and "key sites" network containing only those locations where exposure to *Ae. aegypti* was most likely (houses and schools). Places where participant's spent the most time outside their home during daytime were other residential locations (71% of total time); markets and stores (18%); parks, cemeteries, and recreational areas (3%); and hospitals and health posts (2%). Average degree of a participant (number of locations visited) increased with age from an average (SD) of 2.8 (1.1) for 3-8 yr-olds to 7.1 (4.3) for 45-69 yr-olds. By plotting the in-degree distribution of locations we identified places highly visited by infected individuals (key transmission sites). Our quantitative empiric contact networks indicate that residential exposure can occur beyond 100 m of a person's home and are consistent with the notion that movement of viremic people is a prime driver of rapid DENV propagation in urban environments.

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DENGUE VIRUS TRANSMISSION: THE APPLICATION OF MATHEMATICAL MODELS TO DEVELOP A FRAMEWORK FOR RISK ASSESSMENT

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Development of tools to predict dengue disease, and therefore enable timely intervention, is a topic of intense debate. Risk prediction algorithms should stem from understanding the interaction between different sources of virus transmission regulation that span the environment, human populations, vector populations, and biology of the virus. Location and timing of outbreaks are difficult to predict due to the nature of random events and mobility of humans at large spatial scales. In contrast, probabilities associated with a small outbreak locally escalating into a large epidemic, the rate of reproduction within the local human population, and the spatial extent of transmission related to an outbreak event are aspects of risk assessment that can be quantified statistically. We characterize risk as a space-time probability map that depicts the likelihood of specific events. Thus it is important to (1) establish a set of predictive factors that have a theoretically-measureable role in regulating virus transmission, (2) quantify the relationship between variation among different factors and risk of infection, and (3) develop probability profiles that map space-time risk in relation to changing conditions of transmission. We used a 4-serotype stochastic hybrid SEIR dengue virus transmission model to examine sensitivity of different factors that regulate transmission (environmental, human, vector, and virus strain) for predicting risk. Across simulations, we quantified changes in timing and magnitude of recurrent epidemics under varying conditions of transmission. We examined effects of heterogeneity in relation to dynamics of risk. Heterogeneity has an important cost-benefit relationship in model driven risk assessments. Such models are complex and costly, yet heterogeneity in ecological and epidemiological processes is strongly linked with risk and informs risk prediction. In this presentation, we characterize fundamental relationships between conditions of transmission and assessment of risk for endemic transmission settings with models of varying complexity.

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ENVIRONMENTAL DETERMINANTS OF WEST NILE VIRUS EPIDEMIC IN SOUTH DAKOTA THROUGHOUT 2003 TO 2007

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West Nile virus (WNV) first invaded the Northern Great Plains (NGP) in 2002 and caused a tremendous outbreak in South Dakota in 2003. This study summarized the spatial patterns of human WNV cases in Aberdeen, Sioux Falls, and Rapid City in South Dakota from 2003 to 2007 and investigated the influences of land cover types, hydrological factors, soil conditions, and elevation. We estimated the percentage of urban, open developed space, cropland, grass/hay, and wetland within the neighborhood of geo-coded cases and random controls. We also measured distances from irrigation draw points, hydrological features, and soils susceptible to ponding. The best fitting model was selected according to Akaike's Information Criterion (AIC). Aberdeen has the highest 5-year WNV cumulative infection rate (697.5 per 100,000), following by Rapid City (327.1 per100,000) and Sioux Falls (91.9 per100,000). The statistical models demonstrated the distinctive effects of the environment drivers in the different study areas. Grass/hay (OR=2.8, $p<0.01$) and emergence wetland (OR=1.7, $p<0.05$) predicted the higher risk in Aberdeen. Proximity to soils with high ponding frequency were associated with higher risk in Sioux Falls (OR=1.9, $p<0.01$), however, urban land cover type showed protection protective effect (OR=0.2, $p<0.01$). In Rapid City, risk was associated with both soil conditions (OR=1.5, $p<0.05$) and forest land

cover (OR=5.1, p<0.05) after adjusting for elevation. Our finding indicated that the spatial pattern of human WNV risk can be determined by the environmental variables which represented the unique of topological and hydrological features, soil conditions, and human activity at the local regions. These results suggest that fine-scale spatial models of WNV can be enhanced by adapting them to specific regions.

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GENETIC ANALYSIS OF WEST NILE VIRUS IN THE U.S. SHOWS INCREASING VARIABILITY, 2002-2010

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West Nile virus (WNV) is endemic in the U.S., where it was recognized in 1999, and caused annual outbreaks for 12 consecutive years. By 2010, WNV had caused over 30,000 serious illnesses, including 12,676 neuroinvasive cases and 1,200 deaths reported to the CDC. Viral adaptation to domestic mosquitoes and birds played a major role in the spread of WNV in North America. Reoccurring outbreaks suggest viral adaptation through genetic mutations which have the potential to: alter viral phenotype and virulence; degrade the performance of assays; and affect efficacy of vaccines and potential therapeutic agents. We studied genetic sequences of 140 WNV isolates produced from human plasma, mosquito and bird specimens, obtained from different geographical locations of the U.S. between 2002 and 2010. Genetic sequences were compared with existing sequences in GenBank using Vector NTI. Analyses of phylogenetic relationships were based on parsimony algorithms using MEGA software. In order to expedite surveillance of genetic changes we have developed a microarray-based assay composed of 5 slides containing 1274 overlapping oligoprobes covering the entire WNV genome. Microarray assay validation was performed with 10 previously sequenced WNV isolates. We detected unambiguously all mutations identified in each one of the isolates by traditional sequencing analysis. When compared to the NY99 sequence, results showed increasing genetic variability over the years including deletions and insertion in the 3'UTR. Most mutations were silent transitions (U ~~C~~, A ~~G~~); the number of nucleotide mutations ranged from 20 to 76 resulting in 3 to 17 amino acid substitutions. The 2D RNA analysis of the 3'UTR regions using *mfold* showed that the deletions and insertions identified affect the conformation of some regulatory elements critical for viral replication. Preliminary results of ongoing studies suggest that fixed mutations impact viral phenotype. Further studies are needed to confirm and investigate more phenotypic differences using *in vivo* and *in vitro* models.

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PROCESS-BASED ESTIMATES OF WEST NILE VIRUS TRANSMISSION RISK

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Control programs for West Nile virus (WNV) rely on a variety of surveillance methods focused on mosquitoes and avian hosts to evaluate the risk for pathogen transmission. Intervention strategies are often guided by considering surveillance components individually or by threshold-based risk assessments that provide an overall estimate of human infection risk. These approaches work well for sampled areas, but due to the focal nature of transmission, they are not easily extended to predict risk for unobserved places or times. Here, we evaluated two process-based risk metrics derived from a new dynamic transmission model: the basic reproduction number (R_0) and a novel temperature-driven estimate of the number of vector bites required for transmission (T). The model accounted for several important features of WNV transmission, including the effects of temperature on the virus and mosquito vectors and variation in host competence. For the period since the 2003 invasion of WNV in California, R_0 and T closely tracked the spatial and temporal dynamics of WNV transmission to avian hosts. 5.7% of chickens were seropositive when R_0 was above 1, implying amplification, compared with 1.7% when R_0 was lower. Most (59%) of all seroconversions occurred when transmission was expected to occur within 2-3 mosquito bloodmeals. Mechanistic risk metrics provided earlier warning of the potential for transmission, especially in areas where surveillance was sparse, and were useful for projections to future temperature scenarios that may result from climate change.

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CHARACTERIZATION OF A NOVEL FLAVIVIRUS ISOLATED FROM *CULEX (MELANOCONION) OCCOSSA* FROM IQUITOS, PERU

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In recent years, a number of flaviviruses that replicate only in arthropods have been discovered and characterized. Herein, we describe the isolation and molecular characterization of a novel mosquito-only flavivirus. The novel flavivirus was isolated from *Culex (melanoconion) occossa* mosquitoes collected in 2009 from an urban area of Iquitos, Peru, located in the Amazon basin in the northeastern region of the country. Evidence for a flavivirus was detected by indirect immunofluorescent assay (IFA) in cell culture supernatant of infected C6/36 cells using polyclonal flavivirus group antibodies and confirmed by RT-PCR. In pairwise comparison of the ENV region sequences, the highest nucleotide (47.4%) and amino acid (39.8%) identity was observed with Nounané virus (NOUV). In pairwise comparison of the NS5 region, the highest nucleotide identity was observed with Spondweni virus (65.9%), Iguape virus (IGUV; 65.7%) and Kedougou virus (65.6%); however, at the amino acid level, the highest pairwise identity was observed with IGUV (69.8%), Naranjal virus (69.6%) and Bussuquara virus (69.3%). Phylogenetic analysis using partial ENV and NS5 amino acid sequences revealed this flavivirus forms a clade with NOUV. To investigate the host range of the novel flavivirus, we inoculated a variety of mammalian cells (Vero 76, Vero E6, BHK, LLCMK, and MDCK) with pools of third passage C6/36 isolates and monitored for cytopathic effect (CPE). No CPE was detected, and all mammalian cells lines were negative for flavivirus antigen by IFA and flavivirus RNA by RT-PCR following fourteen days of incubation. We propose that this genetically distinct flavivirus be named Nanay Virus, after the zone of Iquitos, Peru, where it was first detected.

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HETEROLOGOUS NEUTRALIZING ACTIVITY OF JAPANESE ENCEPHALITIS VIRUS GENOTYPE III FORMALIN-INACTIVATED NAKAYAMA VACCINE AGAINST EMERGING GENOTYPE I VIRUS IN TAIWAN

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The circulating Japanese encephalitis virus (JEV) was shifted from genotype III (GIII) to I (GI) virus in Taiwan recently. All commercial JEV vaccines were made from GIII virus and formalin-inactivated Nakayama JEV vaccine was used in Taiwan. To evaluate the homologous and heterologous neutralizing activity of Nakayama vaccine, the vaccinated-children serums were collected in Taiwan, and neutralizing antibodies were measured against JEV GIII vaccine strain (Nakayama), GIII field isolate (CJN-2K) and GI field isolate (TC2009-1). After 4 doses of JEV vaccination, the positive rates of neutralizing antibodies against Nakayama, CJN-2K, and TC2009-1 were 80, 75, and 35%, respectively. However, the neutralizing antibodies were weaning rapidly, because the geometric mean titer (GMT) of neutralizing antibodies persisted 6, 6, and 0 years for against Nakayama, CJN-2K, and TC2009-1, respectively. Immunized with the formalin-inactivated Nakayama vaccine was offered heterologous protection for circulating GIII and GI viruses, when the homologous neutralizing titer (against Nakayama virus) reached above 1:10 and 1:80, respectively. But, among some of low- or non-heterologous neutralization samples, the antibody-dependent enhancement of JEV infection has been observed using undiluted serum samples. This was the first study to evaluate the heterologous neutralizing activity of JEV GIII inactivated vaccine using vaccinated serum samples. Taken together, our study was shown that JEV GIII vaccine offered less neutralizing activity against circulating GI virus, and then might be increased the risk of enhancement of JEV infection.

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TEMPORAL DYNAMICS OF TICKS AND TICK-BORNE ENCEPHALITIS VIRUSES IN A NATURAL FOCUS IN SOUTHERN GERMANY DURING A PERIOD OF TWO YEARS

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Tick-borne encephalitis virus (TBEV) is a member of the genus Flavivirus in the family Flaviviridae. It is transmitted in nature by ticks. So far, the dynamics of TBE natural transmission foci is only partially understood. Different models are used to predict TBEV occurance and risk of infection for humans. In the current study we present data on the abundance of ticks and of TBEV in a single TBE focus duirng a period of two years. Ixodes ricinus ticks were sampled monthly in a standardized way from May 2009 until October 2010. Ticks were sorted according to developmental stage and tested for presence of TBEV using a real time-RT-PCR. Positive tick samples were cultivated in cell culture. The E genes from positive TBEV ticks and positive cell cultures were sequenced and compared to the available sequences. In both years the highest total numbers of ticks were detected in May and June. The total numbers of ticks decreased in June and remained on a stable number for the rest of the year. In 2010 the decrease of tick numbers during the summer was more prominent than in 2009. TBEV infection rates in ticks differed significantly during the two years. While in 2009 eight TBEV positive ticks were from adult stages and one of nine positives came from a nymphal tick, in 2010 two of eight positive ticks were in adults and six positive ticks were nymphs. Although while in 2010 the highest number of ticks was sampled in April, the first positive ticks were only found in May. Ticks as well as TBEV in ticks show significant seasonal abundance. The actual data will help to better understand the dynamics of TBEV in ticks and to predict the risk of infection for humans.

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NON-HOMOLOGOUS INTRA-GENIC RECOMBINATION OF CHIKUNGUNYA VIRUS BUT NOT YELLOW FEVER VIRUS 17D

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Recent phylogenetic analyses of naturally occurring recombinant flaviviruses have raised concerns regarding the potential for the emergence of virulent recombinants either post-vaccination or following co-infection with two distinct wild-type viruses. To characterize the conditions and sequences that facilitate RNA arthropod-borne virus recombination, experiments were performed using yellow fever virus (YFV) 17D and chikungunya virus (CHIKV). Recombinant YFV 17D virus was not detected under any of the experimental conditions examined, despite achieving estimated YFV replicon co-infection levels of ~$2.4x10^6$ in vertebrate and ~$1.05x10^5$ in arthropod cells respectively. Furthermore, YFV 17D specific superinfection resistance was observed in cells harboring a primary infection with wild-type YFV Asibi. Non-homologous recombination was observed for CHIKV within the structural gene coding sequence resulting in an in-frame duplication of capsid and E3 gene. Since this observation demonstrated that the experimental approaches and methods employed were valid and sensitive for recombination detection, we conclude that the generation of viable flavivirus recombinants is extremely unlikely, even in the improbable event of a high level acute co-infection with two distinct YFV genomes.

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MALNUTRITION IS ASSOCIATED WITH ORAL POLIO VACCINE FAILURE IN INFANTS IN BANGLADESH

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Since the 1988 World Health Assembly commitment to global eradication of poliomyelitis, all but 4 countries have interrupted the transmission of wild type 1 and 3 poliovirus. With the subsequent development of bivalent oral poliovirus vaccine, the number of annual cases of poliomyelitis is decreasing. Children with numerous doses of trivalent OPV (tOPV) are still being diagnosed with poliomyelitis in endemic areas. Diarrheal disease has been associated with decreased oral vaccine response, such as tOPV. It is not known if malnutrition is a risk factor for poor oral vaccine response in children from Bangladesh. Our aim is to determine if decreased oral poliovirus vaccine response is associated with covariates of malnutrition. A cohort of 200 infants was followed for 1 year for diarrheal episodes, enteric infections, and malnutrition. Blood samples at 6 and 12 months were tested for neutralization antibodies to poliovirus (PV) types 1,2 and 3. Monthly measurements of height and weight were used to calculate Height and Weight for Age Z-scores. Malnutrition was defined as a Z-score of <-2. Breastfeeding history was obtained from mothers. Sera was tested for C-reactive protein(CRP) and anti-endotoxin, as marker of inflammation, and increased gut permeability, respectively. Results: Average birth weight was 2.67kg (+0.38SD) with 35.5% weighing under 2.5kg; Average Birth HAZ -0.92 and WAZ -1.39. At 1 year of age, the overall sera-response rate was 98.2%, 98.2% and 91% for serotype PV1, PV2, and PV3, respectively. Infants with HAZ <-2 were less likely to seroconvert to PV serotype 2 (P =0.0014) and 3 (P=0.0127). Infants with WAZ< -2 were

more likely to be sero-negative against PV serotype 3 (P =0.0044). Infants who were exclusively breastfed for longer period of time had better seroconversion rates (p-value PV1 0.0597, PV2 0.0506; PV3 0.0048). Anti-endotoxin was not associated with diarrheal events, but there was a negative association with serotype PV2(P=0.0289). Anti-endotoxin at 6 months did predict infants with HAZ<-2 at 12 months of age. No difference found in CRP and decrease vaccine response. In conclusion, by 1 year of age, tOPV underperforms in malnourished infants who received at least 3 doses of oral vaccine. There is a trend for improved vaccine response in exclusively breastfed infants.

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SEROLOGIC CROSS-REACTIVITY OF HUMAN IGM AND IGG ANTIBODIES TO FIVE SPECIES OF EBOLA VIRUS

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Five species of Ebola virus (EBOV) have been identified, with nucleotide differences of 30-45% between species. Four of these species have been shown to cause Ebola hemorrhagic fever (EHF) in humans and a fifth species (*Reston ebolavirus*) is capable of causing a similar disease in non-human primates. While examining potential serologic cross-reactivity between EBOV species is important for diagnostic assays, the nature of cross-reactive antibodies following EBOV infection has not been thoroughly characterized. In order to examine cross-reactivity of human serologic responses to EBOV, we developed antigen preparations for all five EBOV species, and compared serologic responses by IgM capture and IgG enzyme-linked immunosorbent assay (ELISA) in groups of convalescent diagnostic sera from outbreaks in Kikwit, Democratic Republic of Congo (n=24), Gulu, Uganda (n=20), Bundibugyo, Uganda (n=33), and the Philippines (n=18), which represent outbreaks due to four different EBOV species. For groups of samples from Kikwit, Gulu, and Bundibugyo, some limited IgM cross-reactivity was noted between heterologous sera-antigen pairs, however, IgM responses were largely stronger against autologous antigen. In some instances IgG responses were higher to autologous antigen than heterologous antigen, however, we observed strong cross-reactive IgG antibody responses to heterologous antigens among all sets of samples. Finally, we examined autologous IgM and IgG antibody levels, relative to time following EHF onset, and observed early peaking and declining IgM antibody levels (by 80 days) and early development and persistence of IgG antibodies among all samples, implying a consistent pattern of antibody kinetics, regardless of EBOV species. Our findings demonstrate limited cross-reactivity of IgM antibodies to EBOV, however, the stronger tendency for cross-reactive IgG antibody responses can largely circumvent limitations in the utility of heterologous antigen for diagnostic assays and may assist in the development of antibody-mediated vaccines to EBOV.

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BURDEN AND CLINICAL CHARACTERISTICS OF NOROVIRUS DISEASE IN GUATEMALA

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Noroviruses are recognized as a leading cause of diarrheal disease in developed countries. However, limited availability of diagnostics has hindered understanding of their role in developing countries, where most severe diarrheal disease and deaths occur. We therefore sought to determine the disease burden, seasonality, and clinical characteristics of norovirus disease in Guatemala. Centralized local capacity for norovirus diagnostics was established and applied to a population-based surveillance system at multiple government health care facilities. Patients of all ages presenting with acute diarrhea to participating hospitals and ambulatory clinics in two departments, Santa Rosa (October 2007-August 2010) and Quetzaltenango (August 2009-August 2010), were recruited. Demographic and clinical data were collected along with stool specimens for norovirus detection by real-time reverse transcription-polymerase chain reaction. Clinical severity was evaluated using a modified Vesikari score for gastroenteritis on a 21-point scale. Incidence rates were calculated using the catchment area population and adjusted for healthcare utilization rates developed from household surveys. We enrolled 2403 patients with diarrhea in the study, including 528 (22%) hospitalized and 1875 (78%) ambulatory patients; 1460 (61%) were children aged <5 years. Norovirus was detected in 114 (22%) hospitalized patients and 227 (12%) ambulatory patients, with seasonal increases during November-January. Patients infected with norovirus had a median clinical severity score of 6, slightly less severe than that of rotavirus-infected patients (median=8) but more severe than patients with bacterial or parasitic infections (median=4). Overall, we estimate norovirus was associated with 21 hospitalizations, 358 ambulatory visits, and 2261 community illnesses annually per 100,000 population. This study demonstrates that norovirus is a common cause of both moderate and severe diarrheal disease in Guatemala and should inform appropriate clinical management and public interventions for diarrheal disease.

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PRELIMINARY EVIDENCE OF CACHE VALLEY VIRUS INFECTIONS AND ASSOCIATED HUMAN ILLNESS IN WESTERN CANADA IN 2009

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Cache Valley virus (CVV) is a mosquito-borne virus belonging to the family Bunyaviridae, genus Orthobunyavirus that is widespread throughout North America. The virus has been documented to cause congenital defects in livestock but two cases of CVV-associated neurological disease in humans have been reported in the United States. For this study aliquots of sera from Manitoba (MB) and Saskatchewan (SK) residents previously suspected to be WNV cases (febrile and neurological symptoms) but testing negative for WNV during the 2009 mosquito season were tested for CVV antibody. Testing of human sera for CVV antibody was carried out by plaque reduction neutralization tests (PRNT). CVV was propagated in Vero E6 cells and a viral plaque titration was carried out to determine the viral titre per ml. A constant viral dose of 100 plaque forming units was added to serially diluted sera in a PRNT assay to identify CVV specific antibodies. 216 WNV suspect-case sera from SK were initially screened by PRNT at a titre of 1:20 with 9 (5%) of the sera giving CVV specific neutralizing titres of ≥1:20. Sera were further end point titrated with several samples exhibiting significant titres of 40-80 to CVV. An initial testing of 55 sera from WNV suspect cases in MB indentified 9 (16%) patients with CVV antibodies indicating a significant level of virus exposure in this province as well. The application of serological procedures to identify probable CVV infections in symptomatic patients provides preliminary evidence that this pathogen may be contributing to a certain level of exposures and possible illness among patients in MB and SK. Additional surveillance and diagnostic testing is warranted to verify if CVV is associated with disease not only in western Canada but other regions within the country.

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EXPERIMENTAL NIPAH VIRUS TRANSMISSION STUDIES

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Nipah virus first emerged in humans in Malaysia in 1998-1999, during a large outbreak of respiratory disease and encephalitis in humans, causing 276 cases of encephalitis, with 106 fatalities. Nipah virus outbreaks continued to occur in India in 2001 and in Bangladesh 2001 - 2011. Within the outbreaks of Nipah virus occurring in Bangladesh 2001 - 2007 it was estimated that ~50% of Nipah virus cases were due to human-to-human transmission. Nipah virus has been isolated from human urine, saliva, nasal and oropharyngeal secretions suggesting that direct contact with these secretions could result in human-to-human transmission. Epidemiological data suggest that for certain outbreaks a large proportion of Nipah virus patients were exposed to Nipah virus within a hospital setting. Given the potential for nosocomial transmission it is important to understand the mode of transmission of Nipah virus and implement measures to prevent human-to-human transmission in future outbreaks. Three potential modes of human-to-human transmission of Nipah virus could be implicated in human-to-human transmission: transmission via fomites, transmission via direct contact or transmission via aerosols. In this study we assessed of Nipah virus to transmit between humans experimentally through systematic transmission studies using the hamster model.

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CHARACTERIZATION OF A *BRUGIA MALAYI*-ENCODED HUMAN IL-5 RECEPTOR ANTAGONIST (BMIL-5RA) BY RNA INTERFERENCE AND IMMUNOFLUORESCENCE MICROSCOPY

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Understanding the strategies used by helminth parasites to evade the human immune system is of paramount importance if intervention programs are to be successful. We have previously identified a *Brugia malayi*-encoded human IL-5R antagonist (BmIL-5Ra) that not only binds to the human IL-5R, but inhibits human IL-5's ability to signal through its receptor on eosinophils. To further characterize the BmIL-5Ra and to begin to understand its role in both the parasite and mammalian host, antibodies were raised to recombinant BmIL5Ra and used to visualize the expression of the molecule on L3 and other lifecycle stages. To this end, the BmIL-5Ra was localized to the tegument of *Brugia malayi* using immunofluorescence confocal microscopy and immunoelectron microscopy. Constructs were then developed to perform RNAi in *B. malayi* L3 and methods optimized for performing RNAi in these organisms. Using soaking to deliver the RNAi constructs to L3s *in vitro*, we were not only able to show internalization of the double stranded RNA throughout the L3, but we were able to demonstrate inhibition of the BmIL5Ra mRNA (between 1.4 fold and 1.9 fold) in multiple experiments. More importantly, this inhibition caused a quantifiable decrease in the production of excreted/secreted BmIL-5Ra protein as well as a marked decrease in expression on the parasite surface. Thus, we have developed the tools to characterize the function of this protein in the Bm parasite and to assess the role of this protein (and its absence in using RNAi) in modulating IL-5 mediated events in *in vivo* infection models (immunodeficient mice and jird) that in turn should provide important new insights into the host/parasite relationship in human helminth infection.

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ENHANCING IMMUNITY TO *TRYPANOSOMA CRUZI* BY HETEROLOGOUS EXPRESSION OF TLR-LIGANDS

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Chagas disease, caused by the protozoan parasite *Trypanosoma cruzi* affects more than 8 million people worldwide. Infection by *T. cruzi* typically is very 'silent' immunologically and this may contribute to the ability of the infection to become established and persist indefinitely in most hosts. We hypothesize that the relative lack of accessible Pathogen Associated Molecular Patterns (PAMPs) in *T. cruzi* results in a weak activation of innate immune responses and thus the substantial delay in eliciting adaptive immunity. To test this hypothesis, we have heterologously expressed established exogenous PAMPs, namely *Salmonella typhimurium* flagellin (FliC), and *Neisseria meningitides* Porin (NmPorB) in *T. cruzi*. Parasite lines expressing either of these proteins elicited an enhanced innate immune response, as evidenced by their increased ability to activate NFkB/AP-1 reporter cell lines, higher IL-1 induced in macrophages, elevated IL-12 production in IL-12 reporter mice and the earlier generation of relevant serum cytokines in infected BL6 mice. These PAMP-expressing *T. cruzi* lines also elicited a stronger and more rapid *T. cruzi* specific $CD8^+$ T cell response in infected mice, as well as increased IFNγ producing $CD4^+$ and $CD8^+$ T cells. The enhanced immune response generated by PAMP-expressing *T. cruzi* may also have effected a better control of the parasite chronically, as suggested by the higher numbers of central memory$CD8^+$ T cells and reduced parasite load. The strategy of heterologous expression of exogenous PAMPs may be applicable to the generation of improved live-attenuated vaccines for *T. cruzi* and other pathogens.

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MACROPHAGES AND NEUTROPHILS FROM HUMANS AND MICE KILL LARVAL *STRONGYLOIDES STERCORALIS* DURING INNATE IMMUNITY

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The parasitic nematode *Strongyloides stercoralis* (*Ss*) infects 30-100 million people worldwide, yet little is known about the immune response in humans. Previous studies on innate immunity to *Ss* in mice have demonstrated a role for eosinophils, neutrophils (PMN) and complement activation in the protective immune response. The goal of this study was to determine the role of macrophages (MΦ) in innate immunity to *Ss* in humans and mice. Human MΦ were derived from CD34-negative monocytes from G-CSF primed donors and PMN were isolated from the blood of healthy donors. When cultured independently, MΦ and PMN did not kill the larvae; however, larval killing did occur when both human MΦ and PMN were combined *in vitro* in the presence of complement. To examine the role of mouse MΦ in the immune response against *Ss*, bone marrow-derived MΦ were either: 1) cocultured with PMN and larvae *in vitro* or 2) placed in diffusion chambers with larvae and implanted subcutaneously into naïve mice. Larval killing only occurred *in vitro* if both MΦ and PMN were present. In addition, MΦ implanted in naïve mice killed the larvae within 7 days. To determine the phenotype of MΦ during the immune response to *Ss,* mice were infected subcutaneously with larvae and peritoneal exudates cells (PEC) were analyzed by flow cytometry to quantify classically activated MΦ (CAMΦ) and alternatively activated MΦ (AAMΦ). Analysis of PEC from mice with primary infections revealed that both CAMΦ and AAMΦ were present in the peritoneal cavity at levels higher than in control mice. To determine if CAMΦ and/or AAMΦ functioned in killing the larvae, MΦ were stimulated *in vitro* with IL-4 to induce AAMΦ or IFN-γ/LPS to induce CAMΦ. AAMΦ, but not CAMΦ, killed the *Ss* larvae both *in vitro* and after 3 days within diffusion chambers *in vivo*. We conclude from these studies that both human and mouse MΦ,

in conjunction with PMN, kill the parasitic nematode *Ss.* Furthermore, infection of mice with *Ss* results in the induction of AAMΦ which kill the parasite both *in vitro* and *in vivo*.

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EFFECTS OF ANTENATAL MATERNAL PARASITIC TREATMENT ON INFANT ANTIBODY RESPONSE TO *HAEMOPHILUS INFLUENZAE* TYPE B (HIB) VACCINATION IN A MOTHER-CHILD COHORT IN COAST PROVINCE, KENYA

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Antenatal maternal parasitic infections are known to have an effect on fetal immunity. The mechanisms are not fully understood, but it is known that *in utero* exposure to parasite antigens can lead to fetal imbalance of Th1 versus Th2 development that persists into childhood. Studies have shown that schistosomiasis infection has a negative effect on tetanus and BCG vaccination, and malaria infection is associated with decreased response to tetanus, Hib and typhoid vaccination. Positive effects of deworming on vaccine response have been shown with BCG and oral cholera. We sought to determine the impact of treatment of maternal antenatal infection on infant antibody response to Hib vaccination, which is currently unknown. Mothers were tested prenatally and at delivery for parasitic infections, including filariasis, intestinal helminths and malaria. Children had blood drawn every 6 months until 36 months of age, and plasma tested for IgG antibodies against the protective epitope of Hib, poly-ribitol phosphate (PRP), via ELISA. Children were divided into groups by maternal infection status: Uninfected, Infected Treated (prenatal infection; no infection at delivery) and Infected Untreated (infection prenatally and at delivery). 260 mother-child pairs (N=144 Uninfected, N=110 Infected Treated, N=32 Infected Untreated) were analyzed for maternal infection status and infant anti-PRP titers. At 6 months, there was no difference in mean titers between the groups (7.44, 7.61, and 8.32 ug/mL, respectively). At 12 months, the Infected Untreated group had significantly lower mean titers than Uninfected and Infected Treated groups (3.82 v. 6.25 and 6.72 ug/mL, respectively; p=*0.04*). Treatment of maternal antenatal helminth infection is associated with normal infant anti-PRP antibody titers at 12 months, while infants of untreated mothers have markedly lower mean Hib titers. Research is ongoing to determine if this effect persists in older ages. These results suggest that treatment of antenatal parasitic infections may enhance childhood immunity to vaccine-preventable diseases.

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CHARACTERIZING COMPLEXITY IN PRE AND POST-TREATMENT CYTOKINE RESPONSES TO *SCHISTOSOMA HAEMATOBIUM* IN AN ENDEMICALLY-EXPOSED COMMUNITY

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Schistosoma haematobium infection is endemic in sub-Saharan Africa and is associated with cognitive impairment and chronic morbidity. Resistance to *S. haematobium* infection develops in the context of host heterogeneity and exposure to multiple parasite life-cycle stages and may be promoted by anti-helminthic treatment. To investigate how parasite-specific cytokine profiles may contribute to epidemiological patterns of infection whole blood samples were collected from 198 permanent residents (aged 5-84 years) of rural Zimbabwe. Blood was cultured with *S. haematobium* egg and adult worm antigens for 48 hours at 37ºC. Parallel cultures conducted without antigen acted as negative controls. IFNγ, TNFα, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, IL-17A, IL-21 and IL-23 titres were quantified in culture supernatants by enzyme-linked immunosorbent assay (ELISA). Follow-up samples were collected 6 weeks, 6 months and 1 year after a single dose of praziquantel to address the hypothesis that treatment alters cytokine profiles and influences resistance to re-infection post-treatment. Factor analysis was used to identify patterns of cytokine responses before and after treatment and non-metric multidimensional scaling (NMS) was used to identify treatment-induced shifts in host cytokine profiles. Pre- and post-treatment cytokine variations were analysed in the context of host variables via analysis of variance. Data from each participant on their sex, age, co-infection status, residential and anti-helminthic treatment history was collected to inform the analyses. Prior to treatment parasite-specific IL-10/IL-21 responses were positively correlated with infection intensity and IL-17A responses were negatively correlated with infection intensity. These patterns were age-dependent and changed following treatment. A reduced risk of re-infection was associated with elevated schistosome egg-specific cytokine responses. This study presents the most comprehensive analysis of pre and post-treatment *S. haematobium*-specific cytokines to-date and uses novel analytical methods to allow cytokine profiles rather than individual cytokine dynamics to be characterised within a naturally-exposed population. Importantly we have identified a potential role for Th17-associated cytokines in schistosome immunobiology.

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EFFECT OF INDOOR RESIDUAL SPRAYING OF INSECTICIDES ON THE MALARIA SLIDE POSITIVITY RATE IN AN AREA OF HIGH TRANSMISSION INTENSITY IN UGANDA

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There is limited data on the effectiveness of indoor residual spraying (IRS) of insecticide on malaria morbidity in areas of high malaria transmission intensity in Africa. Uganda has recently implemented an IRS program in areas of high transmission intensity through support from the U.S. President's Malaria Initiative. We sought to evaluate the temporal relationship between IRS and the slide positivity rate (SPR) among patients with suspected malaria at one sentinel health facility between Nov. 2006 and Feb. 2011 in the Apac District of Uganda, where the entomological inoculation rate was estimated to be 1586 in 2001. During this period, 3 rounds of IRS were completed. Round 1: March-May 2008 with dichlorodiphenyltrichloroethane (DDT); Round 2: March-April 2010 with alpha-cypermethrin; Round 3: August 2010 with the carbamate Bendiocarb. Over the 52 month observation period a total of 83,829 patients were seen, 41,294 (49%) had suspected malaria, and 77% of those with suspected malaria underwent microscopy. Associations between 6 month periods (with the exception of only 4 months between the 2nd and 3rd rounds) related to IRS and relative changes in the SPR were estimated using Poisson regression after controlling for age and seasonality. The SPR was 45% during the 6 months prior to completion of the 1st round of IRS. The 6 months following completion of the 1st round was associated with a 7% relative reduction in the SPR (p=0.22) compared to the 6 months before completion of the 1st round. The 4 months following completion of the 2nd round was associated with a 12% relative reduction in the SPR (p=0.01) compared to the 6 months before completion of the 2nd round. The 6 months following completion of the 3rd round was associated with a 27% relative reduction in the SPR

($p<0.001$) compared to the 4 months before completion of the 3rd round. In this area of very high transmission intensity, the 2nd and 3rd rounds of IRS were associated with a significant decrease in the SPR. Our analysis will be updated following completion of the 4th and 5th rounds of IRS in 2011.

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IMPACT OF INDOOR RESIDUAL SPRAYING WITH LAMBDA-CYHALOTHRIN ON MALARIA PARASITEMIA AND ANEMIA PREVALENCE AMONG CHILDREN <5 YEARS IN AN AREA OF INTENSE, YEAR-ROUND TRANSMISSION IN MALAWI

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Relatively little is known about the impact of indoor residual spraying (IRS) in areas with intense, year-long malaria transmission such as sub-Saharan Africa. In Malawi, IRS with lambda-cyhalothrin has been applied annually in an area of intense year-long transmission since 2007. We evaluated the impact of IRS on parasitemia and anemia prevalence in children aged <5 years (under-5s) using a cross-sectional household survey conducted in 2009, 6 months after the second IRS spray round. We measured malaria parasitemia and anemia (hemoglobin <11gm/dl) in 899 under-5s and used binomial regression to assess the impact of IRS by comparing under-5s living in a household sprayed with IRS (direct IRS); not sprayed with IRS, but in an IRS area (indirect IRS); and not sprayed with IRS and not in an IRS area (no IRS). In the IRS area, 77% of households reported receiving IRS. Adjusting for bednet use, house construction and socioeconomic status, receiving direct IRS and indirect IRS were significantly associated with a 33% and 46% reduction in parasitemia, and a 21% and 30% reduction in anemia prevalence, respectively.

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EXAMINING THE COMMUNITY EFFECT OF INSECTICIDE-TREATED BED NETS USING SURVIVAL ANALYSIS

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Malaria is a significant cause of child death in sub-Saharan Africa (SSA), causing 715,000 deaths in 2008. Randomized controlled trials across a range of malaria transmission settings have shown insecticide treated mosquito nets (ITN) to reduce child mortality. This reduction in mortality risk occurred not only in children using ITNs but also in children living in villages with high ITN coverage. Mathematical modeling suggests that this protective effect occurs at 50% household ITN usage in the population. Using nationally representative household surveys from 10 countries in SSA we created a retrospective cohort of children aged 1-59 months from complete birth histories, with monthly information on household ITN ownership, proportion of households in the community owning an ITN, age of the child, and malaria transmission season to model the effect of at least 50% of households in the community owning at least 1 ITN on all-cause child mortality. We also included the proportion of children aged 4-59 months receiving 3 doses of diphtheria-pertussis-tetanus vaccine and the proportion of births in the past 2 years delivered at a health facility as indicators of community-level access to healthcare; as well as calendar year, wealth quintile, age of the mother at first pregnancy, education of the mother, and relative birth weight as covariates. Living in a community with >50% ITN coverage is associated with a 32% decrease in the risk of all-cause child mortality. This protective effect is much greater than the 20% decrease associated with household ITN ownership. As expected from previous research, there is a significant community effect when ITN coverage reaches 50% of households in the community- that is children are protected from all-cause child mortality if they live in a community with high ITN coverage, regardless of whether they own an ITN or not. Unprotected children benefit from a reduced vector population, suggesting that investment in other malaria interventions is warranted once ITN coverage exceeds 50%.

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COST-EFFECTIVENESS STUDY ON IMPACT OF LONG-LASTING INSECTICIDE TREATED BEDNETS (LLIN) PROVIDED TO EITHER VULNERABLE GROUPS OR COMMUNITY WIDE ON ANEMIA IN CHILDREN IN SOUTHEAST NIGERIA

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In an integrated lymphatic filariasis and malaria project, LLIN were distributed to all households in 4 local government areas (LGAs) in two states in Southeast Nigeria starting in 2008, with a mop-up round in 2009. Two LGAs received LLIN for vulnerable groups (VG), i.e. pregnant women and under fives; the other two were targeted for full coverage (FC). Anemia rates in children were collected at baseline (2007) and annually for two years by cluster survey. The cost of all inputs in the LLIN distribution were tracked and are included in the analysis, except the costs of the LLINs and the monetary value of the volunteer health workers' time. Together, the cost data and health indicators were used to analyze the cost-effectiveness of the two types of LLIN distribution, from the perspective of funds saved and benefits gained. A total of 171,680 nets were distributed in the FC arm, and 57,251 in the VG arm. Costs were $51,507 and $28,795 in the two arms, respectively. Thus the cost per net distributed was $0.30 in the FC arm, and $0.50 in the VG arm. Net use in all ages increased from 2% (both arms) in 2007 to 62% (FC) and 14% (VG) arms respectively in 2008. The equivalent change in children <5 was 3% (both arms) to 61% and 25% respectively. The cost per 1% increase in net use was $23 ($25 in under 5s) in the FC arm and $99 ($88 in under 5s) in the VG arm. Hemoglobin status in children under 10 improved significantly in both the VG and the FC groups, from 2007 to 2009. The cost per 1% change in hemoglobin (g/dL) for children under 10 was $7,211 for full coverage and $3,311 for vulnerable coverage. The total cost of nets distributed in the VG arm LGAs was lower than that of the full arm LGAs, and achieved a higher percentage increase in hemoglobin. Mean cost to distribute an LLIN was lower in the FC arm, but the cost benefit as measured by improvement in anemia in children was greater in the (targeted) VG. Further analysis of impact and cost effectiveness of these two strategies on malaria prevalence in children and adults is pending.

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AGE-SPECIFIC INCIDENCE OF MALARIA BEFORE AND AFTER SCALING UP OF MALARIA CONTROL STRATEGIES IN BANDIAGARA, MALI

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Recently reported decreases in malaria incidence in many African countries have been attributed to the scaling up of prompt and effective antimalarial treatment using artemisinin-based combination therapy (ACT) and the widespread distribution of insecticide-treated nets (ITN). At a malaria

vaccine testing site in Bandiagara, Mali, ACT was introduced in 2004, and since 2007, ITNs have been distributed free of charge to children after they complete their childhood immunization schedule. We are measuring malaria incidence in an ongoing longitudinal cohort study. Three hundred children aged 0-6 years were enrolled in July 2009 and an additional 100 children aged 7-14 years were enrolled in 2010. Malaria incidence is measured through passive surveillance in the form of expeditious, free medical care and active surveillance through monthly scheduled clinic visits and quarterly blood draws. Ninety percent report ITN use. Preliminary analyses show an incidence of 1.10 malaria clinical episodes per child per season, using a sensitive but non-specific definition of clinical malaria as treatment-seeking behavior with any level of positive parasitemia. Age-specific incidence rates were 0.8, 1.2 and 1.3 for children aged from 0 to 2, 3- 4 and 5- 6 years, respectively. Survival analysis showed that older children experienced malaria illness earlier than younger children. Fifty per cent of children aged 3- 6 years old had their first malaria episode by 8 months from the study start compared to 6 months for children aged less than 3 years (log rank, p= 0.010). Previously, we reported an annual incidence of 1.92 malaria clinical episodes per child per season in 1999-2001, indicating that the implementation of ACTs and ITNs in this setting was followed by a decline in malaria incidence.

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THE ASSOCIATION BETWEEN MALNUTRITION AND THE RISK OF MALARIA IN A COHORT OF HIV-INFECTED AND UNINFECTED UGANDAN YOUNG CHILDREN

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In sub-Saharan Africa, malaria, malnutrition and HIV infection remain major causes of morbidity and mortality in children under five years of age. Few studies have investigated the relationships across malnutrition, malaria and HIV in this age group. Moreover, there is conflicting data on whether or not malnutrition is a risk factor for malaria, and how HIV may modify the malaria-malnutrition relationship. From August 2007 to January 2008, we recruited a cohort of 100 HIV-unexposed, 203 HIV-exposed (born to HIV-infected mothers) and 48 HIV-infected children 6 weeks to 1 year of age living in a high malaria transmission area in rural Uganda. Children were followed up until 2.5 years of age and seen for all their medical conditions in the study clinic. All children were provided with insecticide-treated bed nets. Daily trimethoprim-sulfamethoxazole (TS) prophylaxis was prescribed for HIV-exposed breastfeeding, and HIV-infected children. Height and weight were measured at every visit and stunting was defined as height-for-age z score < -2. Malaria was diagnosed when a child presented with fever and a positive blood smear. The incidence of malaria was compared using negative binomial regression controlling for potential confounders with the measure of association expressed as incidence rate ratio (IRR). The overall incidence of malaria was 3.64 cases per person year. Stunting was an independent risk factor for malaria (IRR 1.20, 95% CI. 1.04-1.39, p=0.01) as was increasing age (IRR=1.41 per 1 year increase, 95% CI. 1.10-1.81, p=0.01), while Urban vs. rural residence was associated with a decreased risk of malaria (IRR=0.42, 95% CI. 0.34-0.52, p<0.001). There was no association between HIV infection and malaria (IRR=0.86, 95% CI. 0.65-1.15, p=0.31), but HIV-infected children were more likely to be stunted (RR=1.50, 95% CI.1.31-1.72, p<0.001. This study suggests that stunting may be associated with an increased risk of malaria regardless of child's HIV-status.

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HEALTH WORKER FACTORS ASSOCIATED WITH CORRECT PRESCRIBING OF ARTEMISININ COMBINATION THERAPY FOR UNCOMPLICATED MALARIA IN RURAL TANZANIA

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Improving malaria case management is partially dependent on health worker adherence to clinical guidelines. We assessed health worker factors associated with correct antimalarial prescribing practices in two sites in rural Tanzania. We conducted repeated cross-sectional health facility surveys and collected information on patient consultations and health worker characteristics. Using logistic regression, we assessed health worker factors associated with correct prescription for uncomplicated malaria defined as prescription of artemisinin combination therapy (ACT) for patients with fever and *P. falciparum* asexual parasitemia on reference blood slide. In this analysis, we included 229 patients with uncomplicated malaria who were seen in a health facility with ACT in stock and 113 health workers practicing in 31 health facilities. Overall, 69% of patients were treated with an ACT. The only health worker factor significantly associated with correct prescription was having 3 or more years of work experience (adjusted odds ratio 6.3; 95% confidence interval 1.7-22.7; p=0.006) while receipt of training on ACT use, receipt of supervision visits, years of pre-service training and availability of job aids were not significantly associated with correct prescription. In conclusion, in this analysis, years of work experience was associated with correct ACT prescription for uncomplicated malaria. Targeted interventions to improve health worker performance are needed to improve overall malaria case management.

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MICRORNAS IN THE PARASITIC NEMATODE *BRUGIA*

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microRNAs are small non-coding RNAs that play key roles in regulating gene expression in animals, plants and viruses. To identify microRNAs in the filarial nematode *Brugia pahangi*, deep sequencing was carried out on small RNA libraries prepared from the third stage larvae or adult worms. Over 120 *Brugia* microRNAs were identified (68% of which were supported by a star strand). Some microRNAs were specific to either stage, but the majority were shared between L3 and adult worm. A small number of additional microRNAs were also identified on the basis of homology based computational searches. Most *Brugia* microRNAs are not conserved in other helminth species. On the basis of library reads, some microRNAs were very highly expressed in the parasite. To further investigate microRNA expression profiles throughout development, microarrays were probed with RNA isolated from six different life cycle stages of *B. pahangi*. Analysis of these has revealed a panel of microRNAs that are either up or down-regulated following the transmission of the L3 from mosquito to mammalian host. In addition a number of microRNAs that differ in expression level between adult males and females were identified. To more fully define the roles of specific microRNAs we are applying existing target prediction programs to the parasite data and adapting techniques reported to allow direct experimental target identification. Candidate parasite microRNA/target interaction will then be verified by transgenic expression using *C. elegans*. Determining if antisense oligonucleotide inhibition approaches are feasible in these parasites is of particular interest and preliminary findings indicate that the uptake of inhibitory oligonucleotides might be achievable in *B. pahangi*.

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TRANSCRIPTOME ANALYSIS OF *BRUGIA MALAYI* LIFE CYCLE STAGES BY DEEP SEQUENCING

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Developing new interventions for the control of parasitic nematodes continues to be a significant challenge. Genomics and post-genomics approaches play an increasingly important role for providing fundamental molecular information about these parasites, thus enhancing basic as well as translational research. Using Illumina high-throughput sequencing, we have undertaken a comprehensive genome-wide survey of the developmental transcriptome of the human filarial parasite *Brugia malayi*. Over 100 million paired-end reads were generated from polyA-tailed mRNA from seven life cycle stages: eggs and embryos, immature MF (of less than 3 days of age), mature MF, L3, L4, adult male and adult female. While deep sequencing data are highly informative in identifying novel transcribed elements and splice variants that help improve the genome annotation, the present study aims to characterize transcriptome changes along the progression of filarial life cycle to further our understanding of the molecular biology of the parasite. Examining the developmental transcriptome profiles of *B. malayi* revealed major transitions in RNA expression from eggs through larval stages to adults. Using statistical approaches, we identified groups of genes with distinct life stage dependent transcriptional patterns and functional categories over-represented in each of these groups. Global transcriptional differences were further evaluated between pairs of stages with particular emphasis on (i) MF maturation, (ii) late larval development, (iii) sex differences, and (iv) intrauterine reproductive processes. Overall, our analysis provides a first comprehensive view of the life cycle transcriptome of *B. malayi*, revealing the dynamics of gene expression during parasite development.

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CROSS-REACTIVITY OR CROSS-SENSITIZATION: MOLECULAR MIMICRY BETWEEN COCKROACH AND HELMINTH GLUTATHIONE S-TRANSFERASES AND ITS IMPLICATION TO THE ALLERGY-HELMINTH INTERFACE

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Although helminth infections have been shown to modulate allergic responsiveness, there are equally compelling data to show that they are associated with the induction of atopy and asthma. Similarities among helminth proteins and allergens are thought to be involved in helminth-driven allergic sensitization. We investigated the molecular and structural similarities between Bla g 5, a major cockroach glutathione-S-transferase (GST) allergen, and the GST of *Wuchereria bancrofti*. These two proteins were found to be 30% identical with a remarkable level of structural conservation based on predicted 3D models. Serological analysis of filaria-infected and -uninfected controls showed that filarial infection was associated with elevation of IgE, IgG and IgG4 anti-Bla g 5, and there was a significant correlation between IgE, IgG, and IgG4 antibodies to Bla g 5 and those to WbGST ($P<0.003$). Pre-incubation of sera from cockroach allergic individuals with WbGST could partially deplete (~ 70%) anti-Bla g 5 IgE, IgG and IgG4 antibodies. Mapping of the IgE binding epitopes for Bla g 5 identified four antigenically relevant epitopes and revealed that the two major N-terminal epitopes were highly conserved in WbGST. Moreover, incubation of sera of filaria-infected patients with the corresponding Bla g 5 peptides inhibited WbGST binding. Finally, mice infected with *Heligmosomoides bakeri* (Hb) developed anti-HbGST IgE and became allergic to Bla g 5 based on skin test reactivity but not to Bla g 4, another important cockroach allergen that has no helminth homologue. Interestingly, when Hb-infected mice were sensitized IP with a mix of Bla g 5 and Bla g 4, there was modulation only of the Bla g 5 allergic response. These data demonstrate that structural similarities can result in allergic cross-sensitization and/or specific cross-modulation depending on the timing of helminth infection and allergen exposure. These findings have important implications for not only understanding the helminth-allergy interface but for the development vaccines to helminth parasites.

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ULTRACONSERVATIVE PROTEINS: BINDING OF MONOCLONAL ANTIBODIES RAISED AGAINST *CAENORHABDITIS ELEGANS* PROTEINS TO SUBCELLULAR COMPONENTS IN THE PARASITIC NEMATODE *BRUGIA MALAYI*

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Few well characterized antibodies are available for studies of subcellular components and organelles in parasitic nematodes. We used immunohistology to explore binding patterns of 21 monoclonal antibodies (mAbs) to known proteins of the model nematode *Caenorhabditis elegans* in the filarial parasite *Brugia malayi*. For 3 mAbs no homologous proteins were identified in the *B. malayi* genome and no structures were labeled in adult *B. malayi*. For another 8 mAbs homologous proteins are present in the *B. malayi* genome, but no staining was detected. Ten mAbs produced distinctive staining patterns in adults as follows: anti-synaptobrevin (SNB-1, a component of synaptic vesicles) labeled the nerve ring of intrauterine microfilariae; anti-EHD1 (RME-1, a marker for recycling endosomes) stained ovaries and early embryos; anti-caveolin (CAV-1) labeled lateral chords and embryonic cells; anti-cytochrome P450 (CYP-33E1) labeled lateral chords, ovaries and testis; anti-HSP-60 (a chaperonin) labeled mitochondria, especially in lateral chords; anti-PAS-7 (part of the 26S proteasome) stained single, non-syncytial cells in the hypodermis and lateral chords; anti-APA-2 (alpha-subunit of the adaptor complex involved in clathrin-mediated endocytosis) labeled hypodermis, lateral chords, ovaries and intrauterine microfilariae; anti-cadherin (HMR-1) labled hypodermis, lateral chords and ovaries; anti-ERM-1 (a cytoskeletal linker in the ezrin-radixin-moesin family of apical membranes) labeled the inner uterus membrane and developing embryos; anti-SAX-7 (an adhesion molecule of plasma membranes) labeled cell membranes in stretched intrauterine microfilariae. These results show that many mAbs that bind to key subcellular structures in *C. elegans* also bind to specific structures in filarial worms. Further work will be needed to confirm whether these antibodies bind to shared epitopes in homologous proteins that have been conserved across some 350 million years of evolution. Epitopes and proteins shared between these distantly related species are likely to be present in other nematodes and critically important in nematode biology.

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THE INVOLVEMENT OF THE *WOLBACHIA* SURFACE PROTEIN FAMILY MEMBERS IN THE ENDOSYMBIOTIC RELATIONSHIP WITH THEIR *BRUGIA MALAYI* HOST

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The molecular basis for the symbiotic relationship between *Wolbachia* and their filarial host *Brugia malayi* remains unknown mystery. There is considerable interest in the filaria - *Wolbachia* relationship due to the dependence of the worms on the endosymbiont for survival and development. Our initial studies indicate WSP-0284, a member of the WSP protein family, potentially plays a role in the symbiotic relationship of the two organisms. The present study was designed to determine whether other members of this family of surface proteins show similar involvement. The genome of *Wolbachia* contains three unrelated WSP proteins and eight additional outer membrane / WSP-like proteins. All these WSPs are members of the outer membrane protein family known to be involved in bacteria-host interactions. We expressed five additional members of the WSP protein family as recombinant proteins and have shown using an ELISA-based assay that 3/5 bind specifically to *B. malayi* crude extracts. Notably, immunoelectron localization studies of two WSP members, WSP-0152 and WSP-0432, indicated that these proteins were not only present on the surface of *Wolbachia* but also in the various host tissues. In particular, anti-WSP-0432 antibodies recognized the protein in the eggshells surrounding the developing microfilaria, and more distinctively in the pseudocoelomic fluid surrounding the adult female worm gonads. To further determine whether WSP-0432 might be involved in the *Wolbachia*-*Brugia* symbiotic relationship and indirectly identify its putative interacting *B. malayi* host protein(s), we used an immunoprecipitation pull down assay followed by mass spectrometry. Interestingly, the most abundant protein that was pulled down was Fructose-bisphosphate aldolase, with many of the others interacting complex proteins also belonging to the glycolysis pathway. The ongoing studies aim to verify the binding of WSP-0432 to their putative interacting host proteins as well as uncovering the possible physiological role of these interactions during the endosymbiotic relationship.

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TRANSCRIPTOMIC AND PROTEOMIC APPROACHES TOWARDS UNDERSTANDING CRITICAL REGULATORS OF MOLTING IN *BRUGIA MALAYI* L3 LARVAE

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Molting of infective L3 larvae into L4s *in vitro* provides a platform to dissect the molecular basis of development. Previously, we provided preliminary microarray data analysis of gene expression early in L3 development to L4. We now provide detailed analyses throughout the L3 to L4 developmental cycle using microarray analysis that reveals a program of ordered gene expression that relate clearly to the lethargus, ecdysis and apolysis of the cuticle. In particular, expression data of >17,000 genes over 10 day period demonstrates clearly upregulated clusters of genes that are likely involved in the transition from vector to human host, in cuticle biosynthesis and its degradation. The most striking observation was the patterns of altered expression of cysteine proteases - cathepsins (CPL-1, -4 and -5) - and serine protease inhibitors (SPN-1, SPN-2) among other genes surrounding the molting process. While we were able to inhibit partially the CPL gene expression in the L3 by RNAi (using dsRNA), the ability to block molting by dsRNA was inconsistent. Nevertheless, chemical inhibition of cathepsin activity inhibited (75-80%) molting of L3 to L4 larvae. Parallel analysis of proteomic data revealed an interesting bias in the cysteine protease expression between L3 and other stages of the parasite. A tight regulation of the collagens and their associated biosynthetic genes could be observed during the molting process. Hierarchical clustering of microarray data and phylogenetic analyses of collagens from *Caenorhabditis elegans* and *Brugia malayi* suggest that Group 2 collagens are specifically regulated during the molt compared to other collagen groups. Interestingly, there were clusters of up- and down-regulated *Wolbachia* genes that also appeared to be developmentally regulated around the molting process. Furthermore, L3-L4 transcriptome data identified ~3000 genes that have not been identified in the whole proteome. Currently ongoing studies using stealth siRNA targeting CPL and serpins and their inhibition of activity will be discussed

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PLEOMORPHISM OF *WOLBACHIA* ENDOBACTERIA IN *BRUGIA* IS INFLUENCED BY WORM AGE AND TETRACYCLINE TREATMENT

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Most filarial species contain *Wolbachia*, obligatory endobacteria that are crucial for parasite development and reproduction. Tetracycline class antibiotics reduce *Wolbachia* loads and affect microfilaria production and worm viability. *Wolbachia* distribution and density vary in different filarial life cycle stages. Past ultrastructural studies of *Wolbachia* in filarial worms suggested that the bacteria have a Chlamydia-like life cycle with small dense bodies as potential infectious forms. We used immunohistology, in situ hybridization, and transmission EM to study effects of age and treatment on the morphology and distribution of *Wolbachia* in L4 and adult *Brugia* parasites recovered from ip-infected gerbils. Parasite material included *B. malayi* recovered at 3, 5, 8, and 12 weeks post infection (wpi), *B. pahangi* recovered at 12 and 112 wpi, and *B. malayi* recovered 5 and 8 wpi after a 2 wk-course of daily tetracycline (5mg/kg) that started at 3 wpi. *Wolbachia* were detected in large numbers in all examined worms with exception of 112 wpi *B. pahangi*, which contained fewer bacteria. Three major forms of *Wolbachia* were observed: (1) Typical bacteria (0.5-1 µm, sometimes dividing) were abundant in the lateral chords and the hypodermis of worms 12 wpi or younger, and they were also observed in the reproductive system of females > 8 wpi. Typical *Wolbachia* were present in lower numbers in treated worms, and they were rare in 112 wpi *B. pahangi*. (2) Large, non-dividing forms (0.9-1.2 µm) were the dominant form in 112 wpi *B. pahangi*, and they were also seen in 12 wpi worms. They were rare in worms less than 12 wpi, and not observed in treated worms. (3) Small (0.2-0.3 µm), electron dense structures were single or grouped (but not clustered). These putative infective bodies were abundant in worms younger than 12 wpi. These forms were absent in 112 wpi *B. pahangi* and only occasionally observed in worms at 12 wpi. Small forms were often present in vacuoles in treated worms, especially at 8 wpi. These results confirm the occurrence of different forms of *Wolbachia* in filarial worms and indicate that the frequency of these forms is related to worm age and external factors such as tetracycline treatment. Further studies will be needed to determine the specific functions of the different morphological forms of *Wolbachia*.

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INCIDENCE OF FATAL LEPTOSPIROSIS - PUERTO RICO, 2010

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Leptospirosis, a potentially fatal infection caused by any of >200 *Leptospira* genus serovars, is endemic in Puerto Rico. Although as many

as 100 cases are reported each year by law to the Puerto Rico Department of Health (PRDH), under-reporting is thought to be high. In January, 2010, the CDC Dengue Branch initiated enhanced surveillance for deaths due to acute febrile illnesses (AFI), which included testing of specimens taken during autopsy of AFI fatalities, an independent review of death certificates at the Vital Registry of Puerto Rico, and comparison of dengue and leptospirosis death rates. Confirmed cases were defined as having: (i) *Leptospira* antigen detected in tissue via immunohistochemistry; (ii) *Leptospira* genome detected by PCR in DNA extracted from tissue or serum; or (iii) detection of anti-*Leptospira* IgM by a private laboratory; suspected cases had "leptospirosis" listed on the death certificate as a cause of or factor contributing to death. Of 101 AFI fatalities that were reported in 2010, 14 (13.9%) were confirmed to be due to leptospirosis; 1 fatal leptospira/dengue virus co-infection was identified. Review of death certificates revealed 9 additional suspected cases; a positive diagnostic test result was found through medical chart review in 1 of these 9 cases. Thus, we identified 15 confirmed and 8 suspected leptospirosis deaths in Puerto Rico in 2010. Of these 23 deaths, the median age was 49 years (range: 19-84), and 18 (78.2%) were male. Cases resided in both rural and urban regions of the island. Medical chart review of all cases is on-going. Although the peak number of deaths occurred in October, lack of comparable leptospirosis surveillance made it impossible to determine if these cases represented an outbreak or baseline levels of disease. In comparison, 38 deaths due to dengue were identified through the same surveillance. In summary, deaths from leptospirosis occurred at a rate of 0.64 deaths per 100,000 residents of Puerto Rico in 2010, which, compared to deaths from dengue, suggests a large burden of disease.

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LEPTOSPIROSIS IN AMERICAN SAMOA 2010 - EPIDEMIOLOGY, ENVIRONMENTAL DRIVERS, AND RISK PREDICTION

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Leptospirosis has recently been identified as an emerging disease worldwide, including in the Pacific Islands. The environmental drivers of leptospirosis transmission vary geographically, and include climate change, extreme weather, land use, international trade, animal reservoirs, and farming practices. We undertook a seroprevalence study to better understand the drivers of emergence in American Samoa. Antibodies indicative of previous infection with leptospirosis were found in 15.5% of 807 participants Three serovars that were previously unknown in American Samoa predominated. Questionnaires and geographic information systems (GIS) data were used to assess behavioural and environmental risk factors. Many risk factors were found to be consistent with previous findings (male gender, outdoor occupations, low income, water exposure), but we additionally demonstrated that there was significant risk associated with living at lower elevation (OR = 1.53), and having higher numbers of piggeries around the home (OR = 2.63). An absolute risk prediction chart was generated using four variables: gender, occupation, knowledge about leptospirosis, and 'piggeries within 250m that have higher elevation than the house'. These variables were chosen because they were significantly associated with leptospirosis, likely to be of practical use for identifying sub-populations at-risk, and for informing potential public health interventions. Our findings suggest that a multi-faceted approach to combating the emergence of leptospirosis is required. Modifying individual risk and managing the evolving environmental drivers of risk need to be considered. These findings are likely to apply to other Pacific Islands with similar climate, culture, lifestyle, and animal-keeping practices. With climate change, the predictions of increasing frequency and severity of cyclones in the Pacific could potentially worsen flooding risk, and exacerbate the disease burden from leptospirosis. Communities need to manage rapidly evolving environmental drivers of risk, and our study will inform their ability to do so.

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EVALUATION OF A DUAL PATH PLATFORM (DPP) ASSAY FOR THE RAPID DIAGNOSIS OF LEPTOSPIROSIS

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Confirmation of leptospirosis by the gold standard MAT requires paired sera and is not widely available. The lack of an adequate and easily implementable diagnostic test hinders effective interventions. We developed a rapid serodiagnostic assay using immunodominant *Leptospira* immunoglobulin-like proteins in a dual path lateral flow platform. This study aims to evaluate the assay's sensitivity, specificity, and reproducibility in the setting of endemic transmission of urban leptospirosis. We measured sensitivity in sera from severe (370) and mild (60) laboratory-confirmed leptospirosis from active hospital and outpatient-based surveillance, respectively, in Salvador, Brazil, and confirmed sera (121) from a reference laboratory in Curitiba, Brazil. We measured specificity in blood bank donors (130) from Salvador, healthy residents (120) of a slum community within Salvador with high endemic transmission of leptospirosis, febrile outpatients (70), and confirmed cases of dengue (65), hepatitis A (64), and syphilis (50). Three blinded evaluators independently scored visual results. Evaluator agreement ranged from very good (kappa 0.86; 95% CI, 0.83-0.90) to excellent (kappa 0.94; 95% CI, 0.92-0.97). Sensitivity was 85% (95% CI, 81-89%) and 61% (95% CI, 42-78%) for acute-phase severe and mild leptospirosis from Salvador, respectively, which was similar to whole-*Leptospira* IgM ELISA (82% [95% CI, 76-86%] and 38% [95% CI, 18-62%], respectively). During the 1st seven days of illness, sensitivity was 77% [95% CI, 66-85%] and 56% [95% CI, 35-75%] for severe and mild leptospirosis. In severe disease convalescence, sensitivity was equivalent (98% [95% CI, 94-99%]) to ELISA (99% [95% CI, 95-99%]). Sensitivity for acute-phase Curitiba sera (58% [95% CI, 46-69%]) was similar to ELISA (66% [95% CI, 55-77%]); whereas it was lower (81% [95% CI, 65-91%]) than ELISA (100% [95% CI, 91-100%]) in convalescence. The specificity was ≥94% for dengue, hepatitis A, syphilis, febrile outpatients, and Salvador blood donors. However, specificity was lower (85%; 95% CI, 77-91%) for healthy residents of a slum within Salvador with high endemic transmission of leptospirosis. The DPP assay performs as well as IgM ELISA for the diagnosis of leptospirosis and can be easily implemented in hospitals where the disease is a major public health problem. However, performance may need to be improved for use in diagnosing milder clinical forms of leptospirosis.

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RICKETTSIOSES: FORGOTTEN CAUSES OF FEBRILE ILLNESSES IN SENEGAL

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Pathogenic rickettsiae, transmitted to humans by arthropod vectors such as ticks are emerging as important causes of acute febrile illness among human populations in Africa. During 2010, we investigated (1) the origin of unknown fever in patients from different districts with a negative arbovirus test result focusing on potential rickettsial infections

and (2) rickettsial strains found in ticks collected from domestic animals in order to assess the risk of these diseases in Senegal. We conducted a laboratory-based jaundice and febrile illnesses study in 2010. Patients' blood samples were assessed for evidence of arboviruses (Yellow-fever, Dengue, West-Nile, Rift-Valley-fever, Chikungunya and Crimean-Congo haemorrhagic fever) and rickettsiae by serological methods. In addition, we assessed ticks collected from domestic animals living in Barkedji (north of Senegal) for the presence of rickettsial agents. A *Rickettsia* genus-specific qPCR assay (Rick17b) targeting a fragment of the17-kD antigen gene consensus sequence was used to screen tick samples and then a *R. africae*-specific qPCR assay (Rafri) was used to test all *Rickettsia*-positive tick samples. Phylogenetic analysis of four rickettsial genes (17-kDa gene, gltA, ompB and ompA) from rickettsial DNA obtained from ixodid ticks was also conducted. All human blood sample tested (196) were negative for arboviruses. Spotted fever and typhus group rickettsiae-specific antibodies were identified from patients with an acute febrile syndrome (20% and 5% respectively). Among 811 specimens collected belonging to six tick species (*Hyalomma marginatum rufipes, H. impeltatum, H. anatolicum anatolicum, H. truncatum, H. dromedarri* and *Rhipicephalus evertsi* evertsi) a total of 174 monospecific pools were constituted and 32% of these pools were positive using Rick17b qPCR assay. All *Rickettsia*-positive samples were negative by Rafri qPCR assay. Phylogenetic analyses of the *Rickettsia* sequences generated from gltA (1124 bp), ompA (591 bp), and ompB (1367 bp) from 7 tick samples demonstrated that they aligned strongly (99.7 to 100%) with *R. aeschlimannii* MC16. These results show the important role of rickettsial diseases among acute febrile illness patients in Senegal. In addition, these results indicate that future work on the clarification of the role of *R. aeschlimannii*, a pathogenic rickettsia for humans, is necessary in north Senegal.

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SENTINELS FOR HUMAN INFECTION: SPOTTED FEVER-GROUP *RICKETTSIAE* IN CANINES

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Rocky Mountain spotted fever (RMSF) is an important tick-borne disease in the United States caused by *Rickettsia rickettsii*. It has been hypothesized that in addition to *R. rickettsii*, other rickettsial species may cause disease and may cross-react with R. rickettsii in serologic tests. There is serologic evidence that other spotted fever group rickettsiae (SFGR), such as *R. amblyommii* and *R. parkeri* may be associated human illness. Additionally, Amblyomma americanum ticks have been found to be infected with *R. amblyommii* and *R. parkeri* in the southeastern United States. Historically, Tennessee has reported one of the highest incidence rates of Rocky Mountain spotted fever in the U.S. Past studies in Tennessee have focused on identifying *Rickettsia* spp. in tick species throughout the state, but little is known about the diversity and prevalence of SFGR in hosts in Tennessee. Sera from 865 canines were collected by veterinarians throughout Tennessee and tested for antibodies to four Rickettsia spp. by enzyme immunoassays (EIA). Indirect immunofluorescent assays (IFA) were performed on all specimens positive by EIA, to confirm specific reactivity to *R. rickettsii, R. amblyommii, R. parkeri*, or *R. montanensis*. Of 275 canine specimens positive for *Rickettsia* spp. by EIA, 41.5% were reactive to *R. montanensis* antigen by IFA, 50.5% were reactive to *R. rickettsii*, and 1.5% were reactive to *R. parkeri*. The seroprevalence of antibodies against *R. rickettsii* was highest in western Tennessee where there is a high incidence of severe RMSF in humans. By using the One Health model with dogs as sentinels, we identified circulation of a variety of rickettsial species that could cause infections in humans. These data suggest that many human infections identified as RMSF in Tennessee may be caused by other rickettsiae. Ensuring the availability of species-specific clinical tests is important to ensure accurate diagnosis of human illness.

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EVALUATION OF DIAGNOSTICS OF *CHLAMYDIA TRACHOMATIS* INFECTION IN KAHE MPYA SUBVILLAGE, ROMBO DISTRICT, TANZANIA: A LATENT VARIABLE MODELING APPROACH

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Trachoma, caused by *Chlamydia trachomatis,* is the leading infectious cause of blindness. Polymerase chain reaction (PCR) is considered to be very sensitive for the diagnosis of *C. trachomatis* infection, but too expensive and time-consuming to be used routinely in a large treatment programme. The method currently used to assess whether populations require control intervention, and to conduct the evaluation of such an intervention, is clinical examination for active disease (Trachomatous Inflammation-Intense (TI) and -Follicular (TF)). We used Latent Markov models to assess diagnostic test accuracy before and after a round of mass treatment with azithromycin in a Tanzanian community (n=956; baseline prevalence of PCR, TI and TF positivity 9.5 %, 13.0 % and 14.6 % respectively), in the absence of a gold standard diagnostic. We defined the true health states as latent statuses of a dynamic latent (unobservable) variable and estimated 3 sets of parameters: 1) transition probabilities which allow for correlation between a respondent's true health state at times *t* and *t*-1; 2) probabilities of response to the 3 diagnostic tools conditional on the latent health status at each time point (i.e. sensitivities and specificities); 3) latent status prevalences (i.e. distribution of the true health states) at each time point. Some key results are: At the individual level, sensitivities and specificities for PCR, TI and TF to identify a) infected and diseased individuals and b) diseased but not infected individuals remained constant during baseline, 2, 6, 12 and 18 months after treatment, for both children <10 yrs and individuals ≥10 yrs. All 3 diagnostic tools were identified as highly specific markers of infection and disease for both age groups. Sensitivities for a) infection and active disease as well as b) active disease without infection, varied among the 3 diagnostic tools at the individual level. Results of this research will help trachoma programme managers identify useful markers to evaluate trachoma control interventions in similar low prevalence settings.

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NATURAL SELECTION IN THE CHOLERA ENDEMIC GANGES RIVER DELTA REGION

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Vibrio cholerae has likely played an important role in human evolution, especially in the Ganges River Delta, the historical and current epicenter of cholera. Observational data support this hypothesis. In particular, individuals with blood group O are at increased risk of severe cholera, and the lowest prevalence of type O in the world is found in the Ganges River Delta. In previous work, we showed that in Bangladesh, cholera aggregates within families independent of blood group, and that the gene *LPLUNC1*, a component of the innate immune system, is associated with susceptibility. Here, we report the first genome-wide study of the non-migrating ethnic group occupying the Ganges River Delta. Using

the Illumina 1M SNP array, we genotyped 36 Bengali mother-father-child trios from Dhaka, Bangladesh. Our results show that Bengalis are a homogenous population group on a distinct branch of the human genetic tree from the 11 populations of HapMap3. Using the Composite of Multiple Signals method, we identified 322 signals of natural selection in the Bengalis (average size 180kb with 2.5 genes; ~163 have 0 or 1 genes). Using INRICH, a new tool designed for genome-wide datasets, we found two especially interesting patterns. First, we repeatedly identified potassium channel genes in strongly selected regions. Second, we identified exceptionally strong enrichment ($p=2x10^{-4}$) for a module of co-expressed genes linked to the gene IKBKG, part of the immunity / inflammation NF-κB complex. Infectious diseases can exert strong selective pressure, and tests for natural selection are a powerful way to find genes influencing susceptibility. We show that, by leveraging massive public datasets and powerful new computational tools, we can identify multiple candidate genes using just 108 individuals. We are now evaluating our candidate genes for association with cholera susceptibility, using transmission disequilibrium testing (TDT) of parents / cholera-affected child trios.

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THE CARTAGENA PROTOCOL IN THE CONTEXT OF RECENT RELEASES OF TRANSGENIC AND *WOLBACHIA*-INFECTED MOSQUITOES

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In the last two years, the first environmental releases of both genetically sterile and *Wolbachia*-infected mosquitoes have been carried out with the goal of controlling dengue fever. Genetically sterile mosquitoes are governed by the Cartagena Protocol on Biosafety - the fundamental document of the United Nations on the international movement of living modified (LM) organisms. Their release provides insight on the suitability of the Protocol for LM mosquitoes. *Wolbachia*-infected mosquitoes are not covered by the Protocol; however their genetic novelty and potential ability to spread across international borders highlight issues relevant to self-propagating LM mosquitoes to which the Protocol does apply. We highlight weaknesses of the Protocol that should be addressed prior to an open release of mosquitoes with gene drive systems intended to spread disease-refractory genes on a wide scale. One weakness, highlighted by recent exports of LM mosquito eggs from the United Kingdom, is that a major section of the Protocol does not apply to LM mosquitoes that are initially intended to undergo laboratory and/or cage studies in the receiving country, and to be released into the environment if these studies are successful. This means that, under the most likely release scenario for any variety of LM mosquito, the exporting country is exempt from performing and financing a risk assessment. Another shortcoming is that, although the Protocol technically applies to non-signatories to the Protocol, these countries may not feel obliged to abide by a Protocol they did not agree to, even if the released transgenes are expected to spread on an international scale. Releases of LM mosquitoes in the Cayman Islands also highlight confusion over the applicability of the Protocol to movements between signatories and non-signatories, which should be clarified. Lessons learned from these releases should guide the Protocol to address the unique biosafety concerns posed by new varieties and future releases of LM mosquitoes.

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WOLBACHIA INFECTIONS OF *AEDES AEGYPTI* AND THEIR POTENTIAL TO CONTROL DENGUE TRANSMISSION

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Wolbachia is a very common intracellular bacterial infection of insects that is maternally inherited and present in up to 70% of all insect species. It does not occur naturally in the major insect vectors of disease however. Recently we have been able to transfer different strains of *Wolbachia* into *Aedes aegypti* where it is maintained and maternally transmitted between generations. It induces a number of effects in the mosquito host including a direct interference effect with dengue viruses, greatly reducing the ability of the mosquito to transmit virus. In recent work we have shown that *Wolbachia* can invade cage populations of wild type *Aedes aegypti* and can also establish itself in wild mosquito populations in open field trials in Australia, opening the way to consider *Wolbachia* as a new tool for the area wide control of dengue.

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SPATIAL-TEMPORAL VARIATION OF *AEDES AEGYPTI* PRESENCE AND ABUNDANCE IN IQUITOS, PERU: IMPLICATIONS FOR DENGUE VIRUS TRANSMISSION

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Spatial-temporal variation of *Aedes aegypti* populations affects both the transmission of dengue (DEN) virus and the effectiveness of vector control efforts. Using extensive *Ae. aegypti* surveillance data collected throughout Iquitos, Peru (1999 through 2002), we evaluated the temporal variation in the abundance of *Ae. aegypti* adult females, and pupae. Results were adjusted for spatial variation in mosquito abundance across the city, as well as differences in surveyor efficiency using linear mixed models. Significant seasonal variation in risk of infestation was observed for both *Ae. aegypti* adults and pupae. Abundance was lowest during July/August and peaked in January. The degree of spatial aggregation within houses also varied seasonally. During periods of low *Ae. aegypti* abundance the population was highly aggregated within houses and became more dispersed when abundance rose. Risk of houses being infested with *Ae. aegypti* adult females was positively correlated with average daily minimum temperature (p=0.02), average wind speed (p=0.0001), and negatively correlated with mean temperature (p=0.02), elevation of the collection site (p=<0.0001) and the water level of the Amazon River (p=<0.0001). The risk of houses being infested with more than 20 pupae was inversely correlated with the river level (p=<0.0001). In contrast the risk of houses being infested with less than 10 pupae was positively correlated with the level of the river (p=0.008). These results indicate that temporal variation in both the abundance and distribution of the *Ae. aegypti* vectors are likely to influence seasonal patterns in DEN virus transmission and disease dynamics.

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INFLUENCE OF HUMAN AND MOSQUITO DENSITIES ON THE PROPAGATION OF DENGUE VIRUS ACROSS HOUSES IN ENDEMIC AREAS

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Heterogeneity in the density of houses, people and *Aedes aegypti* production is a defining feature of dengue endemic cities. However, using this variation to focalize vector control requires a better understanding of the mechanisms through which human and mosquito densities influence viral propagation. At the household level, increased human density favors viral introduction and local propagation through social ties, in addition to increasing the average number of different people exposed to infectious vectors; however, more people reduce the average number

of mosquitoes exposed to infectious people due to frequency dependent mosquito biting. Given *A. aegypti*'s endophilic nature and limited dispersal, increased housing density may favor the infectious exposure of both hosts to each other, whereas increased seroprevalence will reduce only the average number humans exposed to infectious vectors. We used an agent based simulation to explore how the interplay between these processes affects dengue's reproductive rate, given observed ecological variation within endemic areas. Simulations were parameterized based on 7 censuses of *A. aegypti* pupae in 58 patches (2-4 blocks each) of 3 Colombian cities and their respective distributions of residents. Using commonly reported values for survival, biting, dispersal and herd immunity, simulated reproductive rates were comparable to the infection rates observed in a pilot study of clustering of dengue infection in children carried out in the same neighborhoods. Variation across patch-surveys was largely driven by infectious humans infecting many mosquitoes rather than infectious mosquitoes infecting many humans. Increased housing density ameliorated the effects of herd immunity on reducing the reproductive rate. We suggest that given the natural variation in *A. aegypti* densities in areas where domestic water stores generate most vectors, targeting the most productive containers per se is less efficient in reducing the long term transmission rate than reducing vector production in areas with highest human and housing densities.

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THE SPATIAL DIMENSIONS OF DENGUE TRANSMISSION AND EVALUATION OF MOSQUITO INTERVENTIONS

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A significant challenge for the evaluation of new dengue prevention strategies is determining the spatial scale required to detect an epidemiologic effect; i.e., reduction in virus transmission or disease. We use results from entomological and epidemiological studies, across different spatial scales, to examine relationships among mosquito movement, human movement, and dengue virus (DENV) transmission. 25 mark-release-recapture studies indicate that *Aedes aegypti* do not disperse far (typically < 100m) and spatial analysis indicate that a household is the proper spatial scale for measuring *Ae. aegypti* density. When entomological outcomes are the goal, area for treatment is determined more by inter-house variation in mosquito abundance than mosquito movement. Spatial dimensions of DENV transmission can be large and challenging to define due human movement (which affects mosquito-human contact rates and facilitates rapid virus spread) and heterogeneity (spatial and temporal) in virus transmission. Results from contact cluster investigations in Iquitos, Peru (testing blood from people in houses recently visited by a DENV-4 infected person) support geographically dispersed networks of DENV transmission. Attack rates were 17% in DENV positive clusters and 6% in control clusters with more than 50% of contact households >100 m from the index case house and peaks in frequency at 45 m, 265 m, and 1,636 m. Spatially-explicit semi-structured interviews and GPS data-loggers applied to 1,200 Iquitos residents indicate that when not in their own home, most people are in another residence and the average number of sites visited increase with age. We are developing spatially explicit network models to explore the structure of Iquitos human-vector contact networks and their implications for DENV transmission and prevention. Vector control assessments that seek epidemiologic outcomes will need to take human movement into account when selecting the spatial scale for application, or run the risk of obtaining ambiguous results because exposure to virus occurs outside the treatment area.

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EVALUATING ECOLOGICAL NICHE MODELS

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Ecological niche models are being used to predict the geographic range and distribution of medically-important species, including disease vectors, i.e., mosquitoes and sand flies. Ecological niche models predict geographic distribution using species' location records and environmental data (e.g. precipitation, temperature, and land cover). Because of the on-line availability of disease and vector data (e.g., mosquitomap.org) and environmental data (e.g., worldclim.org), models can be assembled quickly. Because most data are not collected for modeling purposes, the data are frequently not adequate to build a good model. While models are traditionally evaluated using statistical methods, other methods of evaluation are equally important such as 1) additional sampling in predicted areas of presence/absence, 2) comparison with disease, host, and other data sets, and 3) comparison of model results to known environmental factors affecting relative population distributions and abundance. This presentation reviews several ecological niche models (published and unpublished) built using the Maxent program for a variety of species, and discusses the methods used to evaluate the models. Models examined were for *Trichinella* spp., *Culex tritaeniorhynchus*, *Aspergillus* (as measured by aflatoxin levels), *Aedes aegypti*, and *Ae. albopictus*. The examples illustrate that steps to reduce clustering in the input data are essential to create good models. Methods to eliminate clustering include removing duplicate records for the same location, additional sampling for under-sampled areas, and resampling the data on a grid. The example models demonstrated that clustered sampling can result in a good to excellent statistical score but poor predictive results. It is therefore critical to evaluate modeling results using several evaluation methods.

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NOVEL AGE BIOMARKERS FOR AFRICAN AND ASIAN MALARIA VECTORS: CHANGES IN PROTEIN EXPRESSION IN HEADS AND THORACES

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Estimation of mosquito age would greatly assist assessments of the efficacy of vector control interventions against malaria. For example, successful vector control with insecticide treated nets (ITNs) and indoor residual sprays (IRS), which reduce transmission by reducing mosquito longevity, would be indicated by a mosquito population dominated by young mosquitoes. However, traditional methods of age grading mosquitoes involve difficult dissections to observe changes occurring in the reproductive system of the females, and only reliably distinguish young nulliparous females from older parous females. In this study, we have shown that mosquito age can also be determined from changes in protein expression occurring in mosquito heads and thoraces.The head and thorax proteome of two cohorts each of *Anopheles gambiae* s.s and *An. stephensi* was compared at different age points (1d, 9d and 17d for *An. gambiae s.s*) and (1d, 9d, 17d and 34d for *An. stephensi*). Proteins were extracted, separated and quantified using Differential in Gel Electrophoresis (DIGE). One way ANOVA was applied to determine

significant changes in protein spot volumes relative to age. For *An. gambiae*, the expression of 6 protein spots changed significantly with adult age for both cohorts (*P*=0.01). However, 14 spots changed with age in both *An. stephensi* cohorts. The abundance of two *An. gambiae s.s* and two *An. stephensi* proteins increased with adult mosquito age while the remainder of the proteins for both species decreased in expression with age. Importantly, two of these spots were shared between the two species. Currently, 2 of these proteins have been identified by mass spectrometry and recombinant forms of these proteins are being used as antigens for antibody production. We propose to develop a cost-effective ELISA age prediction assay from these antibodies to allow for rapid determination of the age of field collected *Anopheles* mosquitoes.

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DESIGNING FOR CHANGE: HOW AN EFFECTIVELY DESIGNED COMMUNITY-BASED BEHAVIOR CHANGE INTERVENTION SUBSTANTIALLY IMPROVED NEWBORN SURVIVAL IN SHIVGARH, INDIA

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It has been shown that simple, low cost interventions such as early and exclusive breastfeeding and keeping babies warm can substantially reduce neonatal mortality in community settings in high mortality regions. Despite this, only modest gains in newborn survival have been made over the past decade. The success of these interventions is contingent upon individual and normative changes in behavior at a community level. Often, these interventions are developed and implemented without taking into account the local realities and cultural context of the communities they are intended to benefit, leading to sub-optimal acceptance and adoption. We developed an essential newborn care intervention package based on the behavior change management framework after an assessment of high-risk newborn care practices, their underlying rationale, and people who had a role in influencing these practices. The intervention was epidemiologically targeted and hybridized scientific evidence with local 'wisdom' and socio-cultural reasoning paradigms in order to improve the understanding and acceptance of the intervention within the community. Further, we harnessed the power of influencers and social networks in order to accelerate normative shifts in behavior. Our approach led to a 54% reduction in neonatal mortality in a period of 16 months, assessed using a cluster randomized controlled trial design in Shivgarh, India. The behavior change management framework can be used to design and implement community-based interventions that are effective from an epidemiological standpoint, and at the same time, suitably adapted to the local socio-cultural context in order to maximize adoption.

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IMPACT OF THE AVAILABILITY OF INTEGRATED COMMUNITY CASE MANAGEMENT ON HEALTH CARE SEEKING BEHAVIOR IN RURAL ZAMBIA

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The provision of integrated community case management (iCCM) for common childhood illnesses by community health workers (CHW) represents a strategy that many developing countries are undertaking in order to reduce mortality of children under 5 years old. In our recently completed cluster, randomized controlled trial, the Zambia Integrated Management of Malaria and Pneumonia Study (ZIMMAPS), we also sought to assess how the availability of iCCM for malaria and pneumonia influenced care seeking behavior. In ZIMMAPS, CHWs were randomized to control [provision of artemether-lumefantrine (AL) to febrile children and referral of children with non-severe pneumonia to the nearest rural health center (RHC) for treatment] and intervention arms [performance of RDTs for febrile children, treatment of RDT-positive children with AL, and treatment of children with non-severe pneumonia with amoxicillin]. We conducted baseline and post-study household surveys on health care seeking practices among women aged 15 - 45 years who had at least one child ≤5 years. The same villages were used in both baseline and post-study surveys. A total of 440 and 441 caregivers were interviewed in the baseline and post-intervention surveys respectively. For children presenting with fever, there was a significant increase in the proportion seeking care from a CHW from baseline to post-study in both intervention [48.3% vs. 81.0%, p<0.0001] and control groups [51.3% vs. 77.9% p<0.0001]. There was a corresponding decrease in the proportion seeking care at RHCs between the baseline and post-intervention surveys in both intervention [35.6% vs. 13.4%, p<0.0001] and control groups [32.5% vs. 13.8%, p<0.0001]. For children presenting with difficult/fast breathing, there was an increase in the proportion who sought care from CHWs from baseline to post-study [50.8% vs. 74.3%, p=0.02 However, in the control group this shift was not found. Our study suggests that the provision of iCCM by CHWS can profoundly influence local health care seeking behaviors. Providing skills and supplies to CHWs is likely to increase the utilization of the services and reduce overload of staff of the public health facilities.

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UTILIZING A PROBLEM BASED APPROACH TO TACKLE GLOBAL HEALTH PROBLEMS: A CASE OF UNIVERSITY STUDENTS IN KIBERA SLUMS

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Kibera is the largest slum in Kenya and in Africa, and is home to 170,070 inhabitants. There are problems that compound access to health care including lack of transport, insecurity, insufficient health facilities and health workers, overcrowding and lack of basic essential amenities like water. The design liberations project assumed a multidimensional problem based approach addressing a broad topic of "Challenges of accessing health care in slum areas". A group of 30 students in university of Nairobi and 30 students from Stanford university from various academic disciplines were brought together in 9 groups. The groups studied the broad topic from perception of nurses, clinicians, policy makers, community health workers, mothers, adults, insurance agencies and the government. The groups used questionnaires and observation techniques to collect data on the broad topic from their identified study informants. The results of the research were then analyzed to develop problem areas which could be addressed by mobile technology and applications developed for these areas. Applications included 'baby bank' to allow pregnant mothers to save money for delivery; 'mnote' a mobile based application to facilitate the work for community health workers; 'mmaji' to assess on the quality and price of water supply in the slum; a pharmacy application to compare and authenticate quality of medicine among other applications. The project successfully addressed needs of the slum residents regarding health, using a bottom-up approach to address problems ranked in hierarchy of needs by key stakeholders within the slum. The project re emphasizes on the need for mutisectoral collaborations as a strategy to address global health problems, need for community participation in developing global health solutions and serves as an example of a simple north south research and technology partnership.

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IMPROVING ACCESS TO SKILLED BIRTH ATTENDANCE IN RURAL AREAS OF NINE SUB-SAHARAN AFRICAN COUNTRIES: RESULTS FROM A PAIR-MATCHED COMMUNITY INTERVENTION TRIAL

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Despite global commitments to achieve the Millennium Development goals (MDGs), progress towards MDG 5 remains slow in sub-Saharan Africa, where virtually no reduction in maternal deaths occurred between 1990 and 2005. We introduced an integrated package of health and infrastructure interventions in high-poverty rural sites in 9 sub-Saharan African countries. A quasi-experimental design and implementation research were used to evaluate the effect of this integrated package of interventions on rates of skilled birth attendance (SBA). Birth histories were collected from 15-49 year old women in randomly selected households in the 9 intervention sites and 9 pair-matched non-intervention sites. Data are available on 3,132 births, including 626 first-time births. The effect of the integrated model is evaluated by comparing the pre-post change in SBA rates in the intervention and non-intervention sites. Implementation research documented the package of interventions including equipped and staffed clinics; roads, water and electricity; referral services; emergency transport; user fees; traditional birth attendant engagement; and incentives. We find that the package was successfully implemented after 18 months in 8 of 9 intervention sites. After three years, SBA rates among all births had increased from 34% to 57% in the intervention sites (p-value=.0001) and from 28% to 42% in non-intervention sites (p-value=.001). Gains in the intervention and non-intervention sites do not differ by a statistically significant amount (odds-ratio=1.3, CI = 0.9 - 1.9). Among first-time births greater differences were observed, with a doubling of SBA rates in intervention sites (39% to 81%, p-value=.004); in non-intervention sites gains were more modest, from 42% to 59% (p-value=.023). Gains in the intervention sites are statistically greater than in the non-intervention sites (odds-ratio= 3.0, CI = 1.1 - 8.6). Despite limited progress towards MDG 5 over the past two decades, these data provide encouraging evidence of recent progress. The implementation of an integrated comprehensive model was associated with dramatic gains in SBA in a relatively short period, particularly among first-time mothers.

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USING GPS DATA TIMESTAMPS AND OTHER MOBILE ICT METHODS TO IMPROVE COMMUNITY-BASED WORKER ACCOUNTABILITY: A RURAL BANGLADESH EXPERIENCE

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Supervisory systems for field-based workers can be difficult and expensive to implement. In contrast to office-based staff, community workers are expected to cover large geographic areas, and only have occasional interactions with supervisors during a work day. Inexpensive Global Positioning System (GPS) units and GPS-enabled smartphones allow novel approaches to performance monitoring. Since 2001, we have conducted randomized controlled trials in a contiguous 436 km2 area of rural northwest Bangladesh, with a field staff of ~750. ~300,000 GIS waypoints are maintained for geospatial analyses and study monitoring. We developed innovative ways of using portable technologies such as Geographic Information Systems (GIS) and mobile phones to monitor and maintain staff accountability and performance across a wide geographic area. We used timestamp signatures embedded in GIS waypoints collected by GPS to assess and map team workers in the field over an 11-week period in 2009. Other examples of performance-assessment methods used in this site include GIS mapping of supplementation adherence, community refusals to participation, and reported vaccination receipt. GPS and location-capable smartphones were also evaluated to quantify the time savings for "expensive" research physicians. Analysis of GPS timestamp data identified poor-performing field workers, who spent a median of 1.89 hours of an expected 5 hour day collecting waypoints. After supervisory counseling, GPS data was used to map worker movements, generating both temporal and spatial data to ensure that expected daily performance benchmarks were being met. For research physician performance, an overall a 32% increase in daily efficiency was achieved using a GIS system, generating 1 extra day's worth of data each week. In conclusion, timestamp and location data from mobile devices, often regarded as uninformative data tags, can improve worker accountability and performance. Innovative use of GPS/GIS technologies can improve program or research efficiency, in addition to allowing for geospatial analyses.

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HEALTH IN HARMONY - HEALTHCARE IN BALANCE WITH THE ENVIRONMENT

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In much of the world, the pursuit of basic human needs and the integrity of the natural environment are in conflict. Loss of biodiversity, poverty, and lack of access to health care are linked and require simultaneous, inclusive solutions. Health In Harmony (HIH) supports Project ASRI in creating a comprehensive community health program that directly links environmental and human health in Indonesia. Five years ago, villages in the project area shared their priorities for improving quality of life. HIH then built upon their suggestions and worked to integrate high-quality, affordable health care with specific strategies to protect threatened rain forests. In the teaching medical clinic, Indonesian physicians and nurses are trained by volunteer physicians; more than 15,000 patients have been served. Public health programs include a team of community health workers and a DOTs program. These vital health care services are connected to several conservation strategies, which serve to link human and environmental health. An incentive program rewards rain forest protection with discounted health care. The reforestation program asks patients to bring in seedlings in exchange for mosquito nets or to pay for their medical care. The organic farm training program has given community members increased crop yields, better nutrition, and a healthier relationship with the natural environment, as well as an alternative method of payment for health care through organic farm labor. In addition, the "Goats for Widows" initiative provides widowed women with two goats to breed and a market for the goats' fertilizer, the organic gardens. Health in Harmony's comprehensive approach to health care provides innovative solutions to preserving the tropical forest, empowering individuals to improve the health of their communities.

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ESTABLISHING INTEGRATED COMMUNITY MANAGEMENT OF MALARIA, PNEUMONIA AND DIARRHEA IN SELECTED TWO LOCAL GOVERNMENT AREAS, AKWA IBOM STATE NIGERIA

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Integrated Community case management (iCCM) of malaria is seen as an essential strategy in achieving Roll Back Malaria coverage targets. Coverage is a key outcome measure but does not help envision the challenging steps needed to establish iCCM. One challenge is deciding and convincing stakeholders to include rapid diagnostic tests in iCCM. Most control efforts have been based within the health services. Formative research in two selected Local Government Areas has shown poor access to malaria interventions is related to distance from health facility, poverty, financial constraints, and perceptions of health services quality. Therefore, iCCM is needed to improve coverage. A team from Jhpiego (affiliate of Johns Hopkins University) and local government health services are piloting an iCCM program based on national guidelines from Nigeria's National Malaria Control Program. This presentation outlines the major organizational, logistical and attitudinal challenges facing the start-up of iCCM. These have included procurement process of rapid diagnostic test kits and anti-malarial drugs, providers' and community poor acceptance of RDTs, community disposal of waste and sharps from RDTs, and providers' and volunteers request for incentives and motivation as program are seen as a burden. Challenges in procurement process include difficulty in sourcing RDTs that come with a complete set of ready to use components. In Onna Local Government communities there is a belief that 'blood of someone alive cannot be buried' because it is believed that blood is life and such burial of blood in a used cassette would mean burying the person alive. To address such challenges we held community dialogue and agreed that used cassettes will be sent to health facility by the CDDs for appropriately burning before burial. This was more acceptable to the community members. We also worked with other malaria partners to identify reliable sources of RDTs. Finally stakeholders meetings helped address reluctance by the health ministry to allow RDT use at the community level. In conclusion we have learned the need for consensus building among partners on roles and extent of services to be provided by volunteers and for community education and dialogue prior to the initial start-up iCCM provision. Without attention to these start-up processes we cannot expect to reach our endpoint coverage indicators.

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IN SEARCH OF PUMP HANDLES: RISK FACTORS FOR DISEASE EARLY IN THE 2010 HAITI CHOLERA EPIDEMIC

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On October 21, 2010, cholera was confirmed in Haiti. Within 1 month, laboratory-confirmed cases were reported from all 10 Haitian departments; Artibonite was one of the earliest and most heavily affected departments. We conducted a case-control study of risk factors for cholera in Artibonite. We enrolled 49 cases - persons ≥5 years old with acute, watery diarrhea admitted to the Cholera Treatment Unit in Petite Riviere between October 25 and November 9, 2010 - and two age- and neighborhood-matched controls per case. We interviewed participants about multiple exposures, including water-related exposures and foods, and conducted household visits. Drinking water was tested for free chlorine as an objective measure of chlorine treatment. Few case-patients (31%) or controls (23%) had an improved drinking water source as defined by WHO. Similar percentages of case-patients (79%) and controls (74%) lacked safe water storage. Although comparable percentages of case-patients (52%) and controls (51%) reported not treating their drinking water before the outbreak, case-patients were significantly more likely than controls to not treat their drinking water during the outbreak (41% vs. 16%, mOR= 3.5, 95% CI: 1.5, 8.7). Stored water from a lower percentage of case-patients than controls had ≥ 0.1mg/liter of free chlorine (27% vs. 39%, mOR= 0.4, 95% CI; 0.1, 1.1). A higher percentage of case-patients (61%) than controls (48%) lacked access to a latrine and, therefore, practiced open defecation (mOR= 2.0, CI: 0.7, 6.1). This study demonstrated that not treating drinking water was the most important factor associated with symptomatic cholera. This finding provides evidence that safe drinking water is a critical need in Haiti. The increase in reported frequency of treating drinking water during the outbreak, particularly among controls, suggests that cholera prevention messaging effectively reached part of the population. The cholera epidemic should galvanize both governmental and non-governmental organizations to address Haitians' need for safe water and adequate sanitation.

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A RAPID QUALITATIVE ASSESSMENT OF CHOLERA RESPONSE EFFORTS, ARTIBONITE DEPARTMENT, HAITI, NOVEMBER, 2010

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On October 21, 2010, Haiti confirmed its first cholera outbreak; Artibonite Department reported 9,694 hospitalized cases and 595 deaths in the first 3 weeks. The Haitian government and non-governmental organizations initiated a communication campaign about cholera, including preparation of homemade sugar-salt solution (SSS) for treatment, through radio, television, and community workers on October 22. Water treatment products and oral rehydration salts (ORS) were distributed. Household surveys in Artibonite revealed that 55% of families had water treatment products and 36% had ORS. A survey of cholera decedents' families suggested that nearly half did not know cholera was treatable. To understand discrepancies between response activities and household preparedness, from November 12-17, we conducted 7 focus group discussions (median size=8 persons) and 5 semi-structured interviews with women regarding cholera prevention and treatment, household water treatment methods, and hygiene. Data were analyzed for dominant themes and concepts using ATLAS-ti software. Analysis revealed that most respondents feared cholera and lacked understanding about cholera prevention, transmission, and spectrum of illness. Discussants expressed a desire for in-depth cholera education. Product distribution events were described as chaotic and stressful, with inequitable distribution of supplies and insufficient education about specific products. Many discussants could describe neither proper dosing for water treatment products, nor correct SSS preparation. Most knew proper ORS preparation, and all discussants noted an insufficient supply. Discussants noted that water treatment products lasted only a few days and replacement supplies were unaffordable. Three weeks into the outbreak, despite intensive communication and product distribution, there were substantial cholera knowledge gaps and insufficient supplies for cholera prevention and treatment. This qualitative assessment rapidly identified correctable deficiencies in cholera response efforts.

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ESTIMATED INCIDENCE OF *VIBRIO CHOLERAE* IN THE CATCHMENT AREA OF A DIARRHEAL DISEASES HOSPITAL IN BANGLADESH

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Evaluating cost-effectiveness of interventions to prevent cholera in Bangladesh depend on estimated community-based incidence. ICDDR,B conducts pathogen specific diarrhea surveillance by testing a 2% sample of patients hospitalized with diarrhoea in it's Dhaka Hospital. Estimating cholera incidence in the catchment area of this hospital using only the hospital data underestimates the cholera burden because many patients seek treatment elsewhere. We estimated the incidence of cholera in Dhaka Hospital's catchment area by adjusting the hospital-based incidence by the proportion of severe diarrhoeal patients within the hospital catchment area who were admitted to this hospital. We defined the catchment area of the hospital as those neighborhoods where more than two-thirds of admitted patients resided. To estimate the proportion of severe diarrhoea cases in the hospital catchment area who were admitted to Dhaka hospital, we conducted a house-to-house survey in randomly selected areas. In the catchment area survey, severe diarrhoea was defined as patients admitted to a healthcare facility, or received intravenous rehydration, or died as a result of frequent loose or watery stools in the previous 12 months. Hospital-based incidence of cholera was calculated by dividing the laboratory confirmed *Vibrio cholerae* cases in the Dhaka Hospital admitted from defined hospital catchment area by the total catchment population. The total population in the hospital catchment area was projected as 9.7 million applying 4.1% annual growth rate to 2001 population census. In the catchment area survey, we visited 38,000 households and identified 895 severe diarrhoeal cases including 2 deaths. The proportion of severe diarrhoeal patients who were admitted to Dhaka Hospital was 0.63 (95% CI: 0.54-0.69). In the hospital surveillance, 339 (18%) cases were positive for *V. cholerae* O1 from March 2010 through February 2011 among the enrolled patients admitted from hospital catchment area during this period. We extrapolated a total of 16,950 *V. cholerae* cases among all admitted patients from hospital catchment area. The population-based incidence of *V. cholerae* was estimated as 275 per 100,000 population (95% CI, 251-317) in the catchment area of Dhaka Hospital. This study provides a credible estimate of cholera incidence in Dhaka, which can be used to assess the cost effectiveness of cholera prevention activities including vaccine.

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PERCEPTION AND PRACTICE ON HANDWASHING LINKED TO CHILD FEEDING IN RURAL BANGLADESH

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Encouraging caregivers to wash their hands with soap before preparing food and before feeding a child may prevent illnesses and improve child growth. This formative study explored community perceptions and practices related to handwashing with soap at these two key times. We conducted this study in 50 rural villages using survey questionnaires, video observations, motivational exercises, in-depth interviews and focus group discussions with caregivers of 6-24 month old children and community members to collect baseline data on handwashing linked to child feeding. Of 350 surveyed respondents, the perceived important methods for ensuring the safety of food for children were to wash utensils with soap and water (61%), wash vegetables, fish, and meat (56%), wash hands with soap before feeding a child (55%), clean the food preparation area (47%), and wash hands with soap before food preparation (40%). Although 18% of respondents reported washing hands with soap before preparing food and 35% during the last child feeding episode, during the video observations, out of 12 opportunities to wash hands with soap before food preparation, participants washed with water alone 5 times, and did not wash their hands 7 times. Out of 27 opportunities to wash hands with soap before feeding a child observed on video, participants washed with water alone 12 times, and did not wash their hands 15 times. The majority of surveyed respondents (60%) cited the unavailability of soap and water near the cooking place as a physical barrier to handwashing before food preparation. In the motivational exercise, most caregivers ranked 'nurture' as the best motivator to encourage washing hands with soap, and 'disgust' as the second best motivator. Although a good proportion of respondents had knowledge that handwashing with soap before food preparation and feeding a child contributes to food safety, this knowledge did not translate into practice. Video observations demonstrated that caregivers in this community do not usually wash their hands with soap before preparing food or feeding a child; the physical absence of soap contributes to a lack of handwashing with soap. Although nutrition and hygiene improvement programs offer opportunities to deliver combined behavior change interventions, to improve handwashing around food preparation in child feeding these approaches will need to address the physical absence of soap, and should employ themes of nurture and disgust.

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ACCOUNTING FOR BIASES IN A HOUSEHOLD WATER TREATMENT INTERVENTION TRIAL IN THE CONGO

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The effectiveness of household water treatment (HWT) devices against diarrheal disease is often measured with intervention trials. It can be difficult to generalize these results outside the study population, and compliance with HWT is often incomplete. We constructed a quantitative microbial risk assessment (QMRA) model to address these issues. The QMRA model simulates a placebo-controlled trial (Boisson et al. 2010, PLoS One 5(9):e12613) of an HWT filter, and accounts for bias due to: A) incomplete compliance with filtration, and B) unexpected antimicrobial activity by the placebo filter. The QMRA model simulates a chain of events in children < 5 years over 12 months with a time unit of 1 day: 1) determine concentrations of 3 marker pathogens (diarrheagenic E. coli, Giardia, and rotavirus) in drinking water; 2) calculate daily doses of pathogens, using pathogen concentrations and water consumption; 3) convert doses to probabilities of infection, using a different dose response function for each pathogen; 4) assign infection to individuals, using probabilities of infection; 5) assign disease, using morbidity ratios. After calibrating the QMRA model to the results of the published trial, the model was used to estimate device effectiveness under different compliance scenarios. Four levels of compliance were considered: low, 65% of children treating 33% of their water; medium, 65% of children treating 67% of their water; high, 65% of children treating 100% of their water; and perfect, 100% of children treating 100% of their water. Compliance was a major driver of effectiveness. Assuming a perfect placebo and low, medium, high, or perfect compliance, the median preventable fraction of reported disease was 14%, 30%, 50%, and 87%, respectively. Adjustment for the imperfect placebo increased the median preventable fraction of disease by 8 percentage points assuming low compliance, but by 22 percentage points assuming medium compliance. The precise level of compliance can greatly affect measurements of HWT effectiveness; such trials should carefully measure compliance.

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ENVIRONMENTAL INDICATORS OF DIARRHEA IN VELLORE, INDIA

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Diarrhea is an important cause of morbidity and mortality in resource-poor settings. This study aims to characterize environmental drivers in transmission of enteric infections in 80 rural and 160 urban households in Vellore, India. Diarrheal episodes were investigated with microbiologic analysis of stool in an ongoing 1-year open cohort study. Information on demographics, hygiene, human/animal interactions, and water sources was collected by questionnaire. Household water contamination was tested using fecal coliform counts. Fly densities were measured in 2 seasons using fly ribbons placed in kitchens. PCR for enteric pathogens were performed on flies. From 8/6/2010- 1/31/2011, there were 91 episodes of diarrhea over 198,795 total person days (PD) with substantial fluctuations in monthly incidence from 0.15 to 0.92 per 1000PD. Fecal coliforms were present in 67% and 74.6% of household water samples from rural and urban areas respectively. Stool pathogens isolated in 24 of 77 (31%) of samples included *E. coli*, *Shigella* spp., *Vibrio* spp., *Giardia spp.*, *Cryptosporidium spp.*, and rotavirus. 43 of 60 (72%) fly samples were positive for pathogens including *E. coli*, *Salmonella* spp., norovirus, and rotavirus. Fly densities were 2.56 times higher during the dry season compared to monsoon ($p<0.001$). The absence of animals in living quarters and indoor latrine use were protective of high fly densities. The adjusted relative risk of diarrhea associated with the 75th% of fly densities was 1.15 [95%CI:1.02,1.29]. Risk factors for increased duration of diarrhea included family size, private well or indoor house-tap use, untrimmed nails, and increased fly densities, while rural living, indoor latrines, no animals in living quarters, and better education were protective. Flies harbored enteric pathogens including norovirus, a poorly documented pathogen on flies. Several modifiable environmental risk factors for diarrhea were identified including water sources, living conditions, hygiene, and fly densities.

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EVALUATION OF HOUSEHOLD MICROBIAL WATER QUALITY TESTING IN A PERUVIAN DEMOGRAPHIC AND HEALTH PILOT STUDY USING THE PORTABLE COMPARTMENT BAG TEST (CBT) FOR *E. COLI*

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The Joint Monitoring Programme of the UN relies on household surveys to determine the kind or source of drinking water supply present. A classification system is used in lieu of actual testing for the microbial water quality due to the unavailability of simple and affordable methods of testing. Demographic and Health surveys (DHS) represent an opportunity to examine household water quality on a large scale if low-cost water quality testing is available. A novel water quality field test was developed by the investigators of this study, field deployed and evaluated. In the new Compartment Bag Test (CBT), a 100-ml water sample is supplemented with a bacteriological medium to support the growth of E. coli, poured into a sterile bag having internal compartments of specified volumes and incubated overnight. If E. coli grows, the water in that compartment turns blue. From the number of E. coli-positive compartments, bacteria concentration is calculated as a Most Probable Number per 100 ml. The purpose of this study was to evaluate the performance of the method in assessing household drinking water quality in the context of a DHS. The pilot study included three regions of Peru with a total of about 750 households surveyed over a 14 week period. Results in the field were compared to the results in reference laboratories analyzing aliquots of the same water samples. Data from the first 10 weeks show that: 1) there was no significant difference between the laboratory (membrane filtration and CBT) and field (CBT) results (Repeated Measures ANOVA $p=0.44$, Friedman Nonparametric Repeated Measures ANOVA $p=0.07$); 2) there was no significant difference between the laboratory or field CBT results of the same water samples; 3) field surveyors and laboratory technicians could easily learn and perform the test. These results suggest that the CBT for E. coli is an effective, simple and affordable method to quantify fecal bacteria in household drinking water as an indicator of safety that can be incorporated into DHS and similar household surveys around the world.

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SCHOOL-BASED MALARIA CONTROL: IMPACT OF INTERMITTENT PREVENTIVE TREATMENT ON MALARIA MORBIDITY AND COGNITIVE FUNCTION IN UGANDAN SCHOOL CHILDREN

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Delivery of intermittent preventive treatment (IPT) of malaria in schools is a promising option for extending malaria control activities to older children. However data on the effects of IPT on the general health and school performance of schoolchildren remain few. We are currently conducting a randomized, single-blinded placebo controlled trial evaluating the impact of two different IPT regimens versus placebo on malaria morbidity and cognitive function among primary school children living in a high malaria transmission area in rural Uganda. In February 2011, 740 children age 6 years to 14 years who met the selection criteria were enrolled and randomized to one of the three study arms: i) Dihydroartemisinin-Piperaquine(DP) given once a school term (every 4 months), DP given once a month, and iii) Placebo. Baseline evaluations included a detailed history, physical exam, cognitive function testing, heamogram (Hb) estimation, stool examination for helminth infections and blood smear exam for presence of malaria parasites. Primary outcomes are the incidence of malaria (fever + parasitemia) and mean cognitive function test scores (using the Raven's matrices and code transmission tests). Secondary outcomes include risk of parasitemia, hospital admissions, adverse events, missed school days, school performance and mean change in Hb levels. An interim analysis of 734 participants completing 3 months of follow up (targeted follow up is 1 year) is presented here. One third (30%) of the children had asymptomatic parastaemia, 37% reported using a bed-net and 9% had helminth infection at baseline. The overall incidence of malaria was 0.08 episodes per person per year; risk of asymptomatic parasitemia was 18% and 31% of children missed at least one school day. Adverse events were reported in 28% of the children and no serious adverse event was reported. Detailed, un-blinded results will be presented at the meeting.

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SCALE-UP OF HOME-BASED MANAGEMENT OF MALARIA BASED ON RAPID DIAGNOSTIC TESTS AND ARTEMISININ-BASED COMBINATION THERAPY IN A RESOURCE-POOR COUNTRY: RESULTS IN SENEGAL

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Effective case management of malaria requires prompt diagnosis and treatment, ideally within 24 hours. In Senegal, case management of malaria in public health facilities was scaled up nationwide with artemisinin-based combination therapy (ACT) in 2007 and rapid diagnostic tests (RDTs) in 2008. Home-based case management may improve access in remote areas with limited access to health facilities. In 2008, 20 villages > 5 km from the nearest health facility participated in a home-based management pilot in which volunteer Home Care Providers (HCP) were trained to manage uncomplicated malaria using RDTs and ACTs, demonstrating the feasibility of integrated use of RDTs and ACTs in the community. Home-based management (PECADOM) was scaled up in 408 villages beginning in 2009. During 2009, 6697 suspected cases were managed by HCP, 92.5% (6198) of whom were tested with an RDT. Among those tested, 34.5% (2144) were positive, 96.1% (2061) of whom were reported successfully treated. Home Care Providers referred 3377 patients to health posts for management: 3324 with a negative RDT, 41 infants < 2 months, 36 pregnant women, and 76 severe cases. There were no deaths among these patients. In 2009 compared to 2008, in districts in which PECADOM was introduced, reported all-cause hospitalizations decreased by 21.7%, malaria hospitalizations by 41.6%, all deaths by 13.2%, and deaths attributed to malaria by 61.4%. Given the simultaneous scale-up of other malaria control interventions, including insecticide-treated nets, during the same period, we used Pearson's correlation coefficient (r) to evaluate the association between HCP/100,000 persons and the absolute decrease from 2008 to 2009 in malaria deaths and hospitalizations per 100,000 persons. We found a moderate positive correlation; for hospitalizations $r = 0.54$ ($p = 0.006$) and for deaths, $r = 0.52$ ($p = 0.008$). Home-based management of malaria including parasitologic testing and treatment based on test results may be a promising strategy to improve the access of remote and rural populations to effective management of uncomplicated malaria and to identify patients needing referral to health facilities, plausibly contributing to a decrease in malaria-related hospitalizations and deaths.

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WHERE COULD INTERMITTENT PREVENTIVE TREATMENT IN CHILDREN (IPTC) BE IMPLEMENTED AND WHAT IS ITS POTENTIAL IMPACT?

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Intermittent Preventive Treatment in children (IPTc) is a promising tool for control of malaria in areas of seasonal transmission. A WHO Technical Expert Group (TEG) recently reviewed evidence for the effectiveness of IPTc with a view to making a policy recommendation. To support the work of the TEG we determined the epidemiologic and geographic situations in which IPTc would be appropriate, and estimate its potential public health impact. First, a series of literature reviews were undertaken to identify monthly malaria data for full calendar years. Several definitions were considered to characterize sites with sufficient seasonality for IPTc implementation. Second, spatial predictors of 'IPTc seasonality' were explored, including rainfall, and climate-driven predictors from a spatial transmission model. Third, based on the continental maps produced by the best spatial predictors, the burden of malaria in under-fives in these areas was estimated using a range of approaches. Lastly, the number of cases that might be averted by IPTc with a range of efficacies and coverage was estimated. Our analyses suggest that the occurrence of 60% or more of the annual number of malaria cases within four consecutive months is the optimal definition of sites suitable for IPTc. The two best predictors for identification of 'IPTc areas' outside the areas for which epidemiological data were available were (i) >60% of the annual rainfall and (ii) >60% of the predicted proportion of total annual transmission from a mathematical model, both within 3 consecutive months of the year. The maps produced by these two predictors identified two potential 'IPTc' areas: (i) large areas of the Sahel and sub-Sahel and (ii) some areas in southern and eastern Africa. We estimate that at least 35 million under-fives live in areas suitable for IPTc. There is considerable uncertainty in making burden estimates, but our lowest estimate suggests that 21.5 million malaria cases and almost 100,000 deaths occur each year in areas suitable for IPTc. Assuming 70% efficacy and 70% coverage, IPTc could approximately halve the malaria burden in these areas and avert many thousands of unnecessary deaths.

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NO REBOUND INCIDENCE OF MALARIA AMONG HIV-EXPOSED CHILDREN AFTER DISCONTINUATION OF TRIMETHOPRIM-SULFAMETHOXAZOLE PROPHYLAXIS IN RURAL UGANDA

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As per WHO guidelines, infants born to HIV-infected mothers should be placed on trimethoprim-sulfamethoxazole (TS) prophylaxis until HIV infection is excluded. We recently showed that TS prophylaxis in HIV-exposed, uninfected children from cessation of breastfeeding until 2 years of age yielded a 39% reduction in malaria incidence. However, it is not known whether prior TS prophylaxis results in a "rebound" malaria incidence. To test this hypothesis we compared the incidence of malaria between 2-4 years of age in the following 3 groups from a cohort of children enrolled at 1.5-9 months of age: 1) HIV-unexposed children (n=88) never taking TS, 2) HIV-exposed, uninfected children (n=80) randomized to stop TS after breastfeeding cessation (median age 10 months, range 6-22 months), and 3) HIV-exposed, uninfected children (n=46) randomized to stop TS at 2 years of age. All children were given a long lasting insecticide-treated net at enrollment. Malaria was diagnosed when a child presented with fever and a positive thick blood smear and was treated with highly efficacious artemisinin-based combination therapy (ACT). Incidence of malaria was compared using a negative binomial regression model controlling for location of residence, the measure of association being an incidence rate ratio (IRR). Incidence of malaria between 2-4 years of age was similar for HIV-unexposed children who had never received TS (5.96 episodes/PY; reference group) compared to HIV-exposed children who received TS through breastfeeding (6.07 episodes/PY; IRR=1.01, p=0.92) and HIV exposed children who received TS through 2 years of age (5.84 episodes/PY; IRR=0.98, p=0.85). Malaria incidence was also similar when comparing HIV-exposed children randomized to stop TS at 2 years of age compared to those randomized to stop after breastfeeding cessation (IRR=1.03, p=0.74). Prior use of daily TS prophylaxis was not associated with a "rebound" incidence of malaria. However, the incidence of malaria between 2-4 years of age was very high in this cohort despite the use of ITNs and prompt and effective ACT.

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ACCESSIBILITY AND AFFORDABILITY OF ANTIMALARIALS IN A RURAL DISTRICT IN KENYA AFTER IMPLEMENTATION OF A NATIONAL SUBSIDY SCHEME

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Poor access to prompt and effective treatment for malaria contributes to high mortality and severe morbidity. In Kenya, it is estimated that only 12% of children receive antimalarials for their fever within 24 hours. The first point of care for many fevers is a local pharmacy or chemist. The role of the medicine retailer as an important distribution point for malaria medicines has been recognized and several different strategies have been used to improve the services that these retailers provide. Despite these efforts, many mothers still purchase ineffective drugs because they are less expensive than effective artemisinin combination therapies (ACTs). One strategy that is being piloted in several countries is an international subsidy targeted at antimalarials supplied through the retail sector. The goal of this strategy is to make ACTs as affordable as ineffective alternatives. The program, called the Affordable Medicines Facility - malaria was rolled out in Kenya in August 2010. In December 2010, we evaluated the affordability and accessibility of malaria medicines in a rural district in Kenya. We did a complete census of all public and private facilities, chemists, pharmacists, and medicine retailers within the Webuye Demographic Surveillance Area. We assessed availability, stock-outs, types, and prices of antimalarials. There are 12 private clinics, 13 public facilities and 84 medicine retailers (registered and unregistered). The average distance from a home to the nearest public health facility is 2km, but the average distance to the nearest medicine retailer is half that. Quinine is the most frequently stocked antimalarial and more shops stocked sulphadoxine pyramethamine (SP) than ACTs. No shops had chloroquine in stock and only 5 shops were selling artemisinin monotherapy. The mean price of any brand of artemether lumefantrine (AL, the recommended first line drug in Kenya) was $2.5. Brands purchased under the AMFm program cost 40% less than non-AMFm brands. Artemisinin monotherapies cost on average more than twice as much as AMFm-brand AL. SP cost only $0.5, a fraction of the price of an ACT. AMFm subsidies have reduced the price of AL, but the price difference between effective and ineffective therapies is still large.

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ACCESS AS A COMPONENT OF EFFECTIVE COVERAGE OF ARTEMISININ-BASED ANTI-MALARIA TREATMENT IN RURAL TANZANIA

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Following declining efficacy of previously used antimalarials, National Malaria Control Programs across sub-Saharan Africa have adopted artemisinin-based anti-malaria combination therapy (ACTs) as first line treatment. Despite excellent efficacy of ACTs, real-world effectiveness of these drugs is reduced by factors related to health systems. The INDEPTH Network's Effectiveness and Safety Studies in Africa (INESS) programme is investigating access, diagnostic targeting, provider compliance, patient adherence and costing to build a complete picture of ACT effective coverage at district scale.This presentation will share findings related to the access component of artesunate-lumefantrine (Alu) in two Health and Demographic Surveillance Sites (HDSS) in rural Tanzania. Linked to the routine HDSS update rounds, continuing surveys were conducted over a one year period to determine access to authorized providers of the official first-line ACTs within 24h and 48 hours of fever onset and reasons for choices and failed access. We surveyed 2,250 individuals resident in 8,874 randomly selected households from two HDSS districts. We analyzed access and care seeking pathways by age, sex, season, household socio-economic status, distance to public and private health providers and community health insurance. We also determined population fever prevalence and parasitemia as well the proportions treated with various other antimalarials in a continuous longitudinal monitoring system. We will share the latest results on access to ACTs from the INESS platform and show how this contributes to understanding effectiveness of ACTs in real-world health systems. We will discuss options on how to improve access and consecutively effective coverage of ACTs in rural Tanzania.

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FORECASTING DEMAND FOR ARTEMISININ COMBINATION THERAPIES UNDER THE AFFORDABLE MEDICINES FACILITY - MALARIA (AMFM)

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The first phase of the Affordable Medicines Facility - malaria (AMFm) is ongoing in Ghana, Kenya, Madagascar, Niger, Nigeria, Tanzania and Zanzibar, and Uganda. By subsidizing the purchase of artemisinin combination therapies (ACTs) to both private and public sector first-line buyers, the program aims to increase widespread usage of ACTs - the only effective treatment for *falciparum* malaria in much of the world - by dramatically reducing the cost to the consumer. Because the global market for ACTs is constrained by the availability of raw materials, long production cycles, and limitations in funding, accurate prediction of the impact of AMFm on global ACT demand is essential to avoid interruptions in supply. However, the scale and uncertainties surrounding this enormous market intervention make development of such a forecast extremely challenging. In 2011, UNITAID sponsored the formation of a consortium tasked with providing periodic forecasts of global ACT demand. The Clinton Health Access Initiative, in collaboration with MIT-Zaragoza and Boston Consulting Group, has developed a novel method for forecasting the uptake of AMFm. This method uses a patient-based approach to estimate the overall demand for antimalarial treatment in the private sector of each country participating in the subsidy. Based on analysis of antimalarial consumption data from previous ACT-subsidy pilot studies, along with data from ACT Watch, DHS/MIS, and in-country support teams, we model the impact that shifts in consumer demand have on AMFm private sector ACT uptake. The forecast estimates AMFm private sector demand for 45.5MM and 68.9MM treatments, comprising 16.5% and 25% of the global ACT market, in 2011 and 2012, respectively. This estimation has been outpaced by demand from first-line buyers during the initial phase of AMFm, indicating that additional market forces are influencing AMFm uptake. Ongoing refinement of this ACT demand forecast, incorporating new data on first-line buyer and consumer behavior, will help to ensure a sustainable supply of these life-saving medications.

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LYMPHATIC FILARIASIS: TREATING A NEGLECTED TROPICAL DISEASE IN THE UNITED STATES

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Lymphatic filariasis (LF) is a mosquito-borne parasitic disease caused primarily by infection with *Wuchereria bancrofti.* Although LF affects an estimated 120 million people in 81 countries, the majority of filarial infections are asymptomatic. However, approximately 40 million persons

are affected by lymphedema or hydrocele, the long-term sequelae of LF. A global effort to eliminate LF is based on annual mass drug administration using antifilarial drugs; in 2008 alone, 496 million people received treatment. In the U.S., there are over 4 million immigrants from LF endemic countries and an estimated 10,000 people have LF. Like many orphan diseases, treatment for LF is readily available in endemic countries, but in the U.S. it is only available through the Centers for Disease Control and Prevention (CDC). CDC has partnered with the Palm Beach County Health Department to implement a pilot program to test and treat immigrants from LF endemic countries. The program aims to offer immigrants the opportunity to be tested and if needed treated within a single clinic visit at no cost to the patient. This pilot program took over three years to establish because both the rapid diagnostic test and the drug treatment although widely used in the global LF elimination program, are not FDA approved and require IRB approval for use in the U.S. A total of 12 clinics within four health centers in the Palm Beach County health department are participating in the program. Each patient from an LF endemic country is offered the opportunity to be tested. Currently, 433 patients have been tested, 425 (98.2%) stated their country of origin was Haiti. Thirty two (7.4%) tested positive and 31 patients were treated; the one untreated patient was ineligible due to pregnancy. The median age of the people positive was 15 years (range 6 - 40). No adverse events were reported. The staff and patients are very supportive of the program. This program provides access to LF treatment to a U.S.-based population that was exposed in the their countries of origin. This program could serve as a model for other orphan parasitic diseases.

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INCIDENCE AND GEOGRAPHICAL DISTRIBUTION OF SERIOUS ADVERSE EVENTS FOLLOWING MASS ADMINISTRATION OF IVERMECTIN IN CAMEROON FROM 1999 TO 2009

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Mass treatment with ivermectin started in Cameroon in 1987, followed by community-based ivermectin treatment and Community Directed Treatment with Ivermectin (CDTI) implemented by the National Onchocerciasis Control Program (NOCP) in 1999. In 1999, a cluster of serious adverse events (SAEs) was reported in an area endemic for loiasis. This prompted the institution of a surveillance system in Central Africa to promptly address SAEs.The present study evaluated the annual incidence and risk factors of post-ivermectin SAEs from 1999 to 2009 in Cameroon. Treatments data were obtained from the Cameroon NOCP. Medical files of subjects presenting with SAEs were analysed, and geographical coordinates of their communities of residence were collected. A total of 9,057,076 treatments was administered in the 38 health districts from 1999 to 2009. During this time, 382 SAEs were recorded, giving a cumulative incidence of 4.2 cases/100,000 treatments. The outcome was fatal in 11 cases for a mortality rate of 1.2 deaths per million treatments. The annual incidence of SAEs decreased from 8.6 cases/100,000 in 1999 to 0.9 cases/100,000 in 2009. Nearly all (95%) of the SAEs occurred following the first ivermectin treatment. A mean period of 32.9 hours (ranged 2-168 hours) elapsed between the treatment and the first symptoms. Nearly all (91.6%) of the subjects with SAEs were found to have *Loa loa* microfilariae in post-treatment blood samples. Furthermore, all SAEs occurred in regions predicted to be highly endemic for loiasis. This study confirms previous data demonstrating that loiasis is the main risk factor of SAEs following CDTI and that the risk is greatest after the first treatment. The current maps predicting Loa loa endemicity will be useful in the identification of at-risk areas, and will be necessary to guide the NOCP in Central Africa during the planning and implementation of CDTIs for onchocerciasis and lymphatic filariasis control in untreated areas. In areas treated for some years, it may be helpful to test ivermectin naive individuals for *L. loa* before treatment.

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THE HISTOPATHOGENESIS OF IVERMECTIN-INDUCED LOIASIS-ASSOCIATED PATHOLOGY IN PRIMATES

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Understanding the pathogenesis of the severe adverse effects that occur in patients with high circulating loads of *Loa loa* microfilaria can suffer when treated with ivermectin has been a major goal of those associated with the distribution of this important drug for the control and elimination of onchocerciasis and lymphatic filariasis in areas of Africa endemic for loiasis. The tissues examined from splenectomized baboons carrying very high loads of circulating microfilariae (>100,000 mf/ml) revealed a number of tissue changes and clinical changes in these animals consistent with those reported in humans suggesting that the baboon model is a useful model for studying and understanding the pathogenesis and can allow for development of therapeutic approaches to managing the human condition. These changes in the baboons after ivermectin treatment included parasitic thrombi, fibrin deposition and damage to vascular endothelium. Petechial hemorrhages were commonly found at autopsy in the CNS and other tissues, as was acute damage in the tissues surrounding these vascular lesions. Chronic inflammatory responses were seen in the liver that occasionally were associated with microfilarial death and tissue eosinophilia; these are believed to be inherent to the long term presence of high loads of Loa loa and not due to the ivermectin treatment. All of the 12 animals studied shown evidence of considerable regeneration of splenic tissues, new organs that were very actively involved in degeneration and destruction of microfilariae. Histological evidence of dermal Mazzotti reactions were also common post ivermectin therapy. These finding suggest that the adverse clinical responses seen after ivermectin treatment in hosts with high levels of circulating microfilariae are vascular based lesions and that there is a tendency for these involve the central nervous system.

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IMPACT OF A COMMUNITY-BASED LYMPHEDEMA MANAGEMENT PROGRAM ON PERCEIVED DISABILITY, PRODUCTIVITY AND QUALITY OF LIFE AMONG LYMPHEDEMA PATIENTS IN ORISSA STATE, INDIA

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Lymphatic filariasis (LF) infects an estimated 120 million people worldwide, causing lymphedema and hydrocele in over 40 million. India comprises over 40% of the world's LF burden, with millions of people in need of lymphedema management. A community-based lymphedema management project in Orissa State, India, was begun in 2007 by the Indian non-governmental organization, Church's Auxiliary for Social

Action (CASA) in consultation with the Centers for Disease Control and Prevention, and has enrolled and treated over 21,000 lymphedema patients. To assess the impact of this program on disability, productivity, and quality of life, a random sample of 375 patients was followed over their first 12 months of enrollment. Each patient was interviewed at enrollment and at 1, 2, 3, 6, and 12 months thereafter. Perceived disability and quality of life were measured using the WHO Disability Assessment Schedule II (WHO DAS-II), a well-validated, 36-question tool, which assesses 6 domains of functioning: cognition, mobility, self-care, getting along with others, life-activities, and participation in society. Mean disability scores (simplified, non-weighted scoring) at each time point were compared using paired T-tests in SAS 9.2. Statistically significant decreases in disability were observed in all 6 WHO DAS-II domains within 3 months of enrollment, and were most pronounced at 6 months post-enrollment. At 6 months, the decreases in self-reported disability (compared to baseline) were 17% for cognition (P<.0001), 6% for mobility (P=.01), 13% for self-care (P<.0001), 21% for getting along with others (P<.0001), 7% for life activities (P=.01), and 15% for participation in society (P<.0001). At 12 months, the magnitude of each change was smaller than at 6 months, but remained statistically significant for all domains except mobility (3% reduction compared to baseline (P=.13)). When asked how many days in the past month they were totally unable to attend to their daily work due to their lymphedema, patients reported 3.3 fewer days lost at 6 months (95% CI 2.4-4.3) and 2.4 fewer days lost at 12 months (95% CI 1.3-3.2). These data demonstrate a beneficial impact of this lymphedema management program on quality of life and productivity, and emphasize how community-based lymphedema management can provide psychological support and increase productivity for those suffering from lymphedema.

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TOGO'S NATIONAL LYMPHOEDEMA MANAGEMENT PROGRAMME: EVALUATION OF PROGRESS OF PATIENTS AFTER THREE YEARS

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In 2007, Togo's Ministry of Health, with technical assistance from the Centers for Disease Control and Prevention, started a National Lymphoedema Management Programme. In each health structure in the country, including non-endemic districts, at least one person was trained in lymphoedema management following the recommendations from the World Health Organization. The aim was to train patients in self management of their lymphoedema. A cohort of patients was followed each year during three years to evaluate the impact of the program on the physical well-being and quality of life of the patients. Data were collected annually. The primary indicators used to evaluate the project include included changes in treatment behavior, incidence and duration of acute attacks, and quality of life. Data for 107 patients of the 166 originally selected by convenience sample in 6 districts were available for paired analyses of 2007 and 2010 responses. Fifty three percent of the patients were female, and the median age was 46 years (6-90). In 2010, 95 % of the patients followed were using at least one promoted treatment compared to 25 % in 2007 (OR=38.5, 95% CI: 11.33, 233.4). Patients were also more likely to report current use of each of the three promoted treatments -limb elevation (18% to 77%, OR=16.75, 95% CI: 6.67, 54.08), exercise (4% to 79%, OR=81.00, 95% CI: 16.05, 1638.00), and washing (10% to 93%, OR=89.00, 95% CI: 17.67, 1798.00). Patients were less likely to report no current lymphoedema treatment use (31% to 3%, OR=26.47, 95% CI: 0.010, 0.22). However, between 2007 and 2010, there were no significant improvements in patient self-sufficiency (as measured by activities limited by lymphoedema symptoms) or stigma There was no significant change in median number of acute attacks per year (2007 median: 2, 2010 median: 2, p=0.15). However, there was a significant decrease in median duration of acute attacks (2007 median: 6 days, 2010 median: 4 days, p=0.05). Although we have heard very positive feedback from the patients themselves, according to our survey, very little improvement was made on patient outcomes, although a high rate of loss to follow up limits our analysis and better tools are needed for quality of life assessments. We recommend that to assess the impact of national lymphedema management programs on patient outcomes, objective measures are needed to supplement self-reported data.

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AFRICAN PROGRAMME FOR ONCHOCERCIASIS CONTROL: IMPACT AND COSTS BY 2010

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Onchocerciasis causes a considerable burden of disease in Africa, mainly due to skin and eye disease. Since 1995, the African Programme for Onchocerciasis Control (APOC) has coordinated annual mass treatment with ivermectin in endemic countries. In this study, we estimated the effect of APOC on population health and the associated costs up to 2010. Using data from pre-control mapping studies, we estimated the pre-control prevalence of infection in APOC areas. Next, using data from APOC's mass treatment records and the micro-simulation model ONCHOSIM, we estimated the decline in infection, blindness, visual impairment and severe itch between 1995 and 2010. The associated burden of disease was expressed in disability adjusted life years (DALYs). Data on costs made by APOC, non-governmental development organizations, beneficiary governments and the Mectizan Donation Program (MDP) were obtained from reports, where available. We estimated that between 1995 and 2010, mass treatment with ivermectin caused a considerable decline in the overall prevalence of infection with O. volvulus (from 39% to 14%), troublesome itch (from 14.5% to 3.0%), visual impairment (from 1.0% to 0.7%) and blindness (from 0.4% to 0.2%) in APOC areas. We estimated that 1.5 million DALYs were lost due to onchocerciasis in 1995. Due to population growth, this loss would have been 2.2 million DALYs in 2010 if there had been no mass treatment. However, in 2010 only 0.7 million DALYs were lost. Overall, we estimated that APOC has cumulatively averted about 8.8 million DALYs due to onchocerciasis up to 2010. The total associated costs were estimated at about US$2.4 billion (US$2.2 billion for MDP only). In conclusion, APOC has had a great impact on population health in Africa, at a relatively limited cost.

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ELIMINATION OF ONCHOCERCIASIS TRANSMISSION IN MT. ELGON FOCUS OF EASTERN UGANDA HAS BEEN ATTAINED

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The Mount Elgon onchocerciasis focus is a highly isolated, having an area of 250 km^2, and located in eastern Uganda on the border with Kenya.

The vector is *Simulium neavei s.s.*, and its larvae develop in a phoretic association with the fresh water crab *Potamonautes granviki*. Annual ivermectin mass drug administration (MDA) was launched in Mount Elgon in 1997. In 2007, ivermectin MDA was changed to twice yearly treatment after the government of Uganda moved from a policy of control to elimination, and intensified interventions. MDA coverage has been over 90% of eligibles since the 1997 and was unaffected by the switch to twice per year treatment. In early 2008, vector elimination activities using the larvicide temephos (Abate®) at 0.2-0.4 ppm were applied once a month at identified breeding sites for a year, then every other month for a period of 6 months, and then stopped. Baseline crab trapping and examinations for *S. neavei* infestation and black fly landing catches were conducted monthly since 2007. Sentinel village (SV) examinations with skin snips to measure microfilardermia (mf) prevalence were conducted in 1994 and 2011. Serologic testing (OV16 antibody) of children below the age of 14 years was conducted in 2010. The Abate campaign drastically reduced crab infestation with larvae and pupae of *S. neavei* from 30.2% pre-control in 2007 to 0% by September, 2008, and has remained so since. Adult fly biting rate reduced from 5 flies per man hour in 2007 to 0 in July 2008, and has remained at zero through April 2011. Mf prevalence in SVs dropped from over 50% in 1994 to 0.003% in 2011. Only one child out of 3051 was positive for antibody. We conclude that transmission of onchocerciasis has been interrupted, recommend halting of all interventions, and commencement of post treatment surveillance activities.

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AEDES AEGYPTI SALIVARY PROTEINS MODULATE DENDRITIC CELL IMMUNITY TO DENGUE VIRUS INFECTION

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Hematophagous arthropod saliva has been shown to possess a variety of effector functions that facilitate the acquisition of a blood meal. Mosquito saliva contains molecules with anti-inflammatory, anti-haemostatic, and immuno-modulatory capabilities. Arbovirus-infected mosquitoes expectorate saliva and virus immediately prior to blood feeding and this saliva may have the potential to aid the establishment of arbovirus infection within the vertebrate host. One such molecule in the saliva of *Aedes aegypti*, the primary vector of dengue virus (DENV), is Aegyptin, a protein shown to inhibit platelet aggregation. The effects of Aegyptin on the immune response profile of primary human monocyte-derived dendritic cells (moDCs) were examined using multiplex cytokine immunoassays. Aegyptin in combination with DENV type 2, strain 16803, demonstrates increased production of the anti-inflammatory cytokine IL-10 at time points beyond 12 hours, raising secretion levels in relation to those of mock infected or virus-only infected cells. The increase in IL-10 levels positively correlates with increases in moDC secretion of the chemokine IP-10 in groups treated with this combination of Aegyptin and virus. Further, the production of IL-4, a cytokine necessary in the shift toward a TH2 response, decreased over time, with particularly notable decreases in virus infected groups as compared to controls. Lowering levels of IL-4 taken in conjunction with the increases in IP-10 and IL-10 secretion levels could indicate that DENV in combination with Aegyptin creates an environment more permissive to infection, where immune cells suspected to serve as DENV replication sites, such as dendritic cells and macrophages, are being recruited to the site of infection (IP-10), suppressed in their antiviral, TH1 functions (IL-10), and minimally activated to a TH2 immune state (IL-4). Further characterization of the roles of other specific mosquito-expectorated proteins in the establishment and course of arboviral infection is needed.

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REDUCED ANTI-HEMOSTATIC QUALITIES OF *AEDES AEGYPTI* SALIVARY EXPECTORATE FOLLOWING DENGUE-2 VIRUS INFECTION

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Dengue virus is mainly transmitted by *Aedes aegypti* mosquitoes and the virus must disseminate into the salivary glands in order to be transmitted to the vertebrate host via salivation during probing and/or feeding. The saliva contains a diverse cocktail of pharmacologically active compounds that are deposited with the virus in bite site of the vertebrate host. This is where the mosquito's saliva can alter the local environment, perhaps in a way that facilitates the establishment of an infection. In order to determine if dengue virus is altering the composition of that cocktail by altering the expression of various salivary components, we have analyzed the protein composition of *Ae. aegypti* saliva in bloodfed needle-inoculated dengue-2 infected mosquitoes and uninfected bloodfed control mosquitoes via 2-D gel electrophoresis. Using naturally-expectorated saliva, the resultant salivary proteins were precipitated, desalted, and reconstituted for further proteomic analysis. We have found a global down-regulation of the majority of the proteins in the saliva, with the exception of the most abundant proteins, the various isoforms of D7 and apyrase. Previously identified proteins such as C-type lectin 1 and salivary serpin 4 were down-regulated 10-fold in infected saliva, and a previously unreported low-density lipoprotein receptor was found to be secreted and also reduced 10-fold in infected saliv*A. aegypti*n, adenosine deaminase, C-type lectin 2, and an inosine-uridine preferring nucleoside hydrolase were reduced 5-fold. The majority of these down-regulated proteins have been shown to be involved in the mosquito's anti-hemostatic response, perhaps leading to an increase in viral inoculum by the infected mosquito to compensate for the increased hemostasis or increased viral dissemination due to feeding interruptions. This research indicates the need to not only review the components of mosquito saliva that are being inoculated alongside the virus, but also the effect the virus has on the composition of those components in the saliva.

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VIRAL AND IMMUNOLOGICAL DETERMINANTS OF DENGUE VIRUS FITNESS AND DISEASE SEVERITY

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The relative contributions of viral evolution, pre-existing immunity, and host genetic factors to dengue virus (DENV) fitness and disease severity remain unclear. In Managua, Nicaragua, we observed an abrupt increase in disease severity across three epidemic seasons of DENV-2 transmission in two independent studies of pediatric dengue. Sequence analysis of full-length genomes of viruses isolated from patients identified a genetically distinct clade of DENV-2 circulating in later epidemic seasons. Viruses from the replacing clade replicated more productively in human and mosquito cells and had longer-lasting viremia in patients, which supports the emergence of a more-fit virus. However, association analyses revealed that the abrupt increase in disease severity occurred across years, irrespective of clade. Analysis of immunological profiles in children from both studies demonstrates a role for pre-existing DENV immunity, as DENV-3 immunity is associated with severe disease in our cohort study. Further, both waning immunity to DENV-1 and a specific interaction between DENV-3 immunity and viruses from the replacing clade appear to be playing a role in

increasing severity across seasons. In sum, our data demonstrate that it is the interplay between viral genetics and host immunity that is the major driver in determining risk of severe dengue disease.

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A MODEL OF LETHAL DENGUE VIRUS 2 INFECTION IN C57BL/6 MICE DEFICIENT IN THE IFN-A/ß RECEPTOR

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The four serotypes of dengue virus (DENV1-4) cause dengue fever and dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS), the most prevalent arthropod-borne viral diseases in humans and a major public health problem worldwide. Animal models for dengue are needed to study the mechanisms underlying disease pathogenesis as well as the complex immune response to primary and secondary DENV infections. We have previously demonstrated that a mouse-adapted DENV2 virus, D2S10, caused mortality after intravenous infection at a high dose in 129/Sv mice doubly deficient in interferon (IFN)-α/β and IFN-γ receptors (AG129). Here, we characterize a more virulent DENV2 strain, D220, that was obtained via ten alternate passages of D2S10 between mosquito cells and AG129 mouse serum. D220 is lethal after intravenous infection of AG129 mice with a 10-fold lower dose than D2S10. In C57BL/6 mice that are deficient in only the IFN-α/β receptor (A-B6), D220 is 80% lethal with 10^6 plaque-forming units (pfu); in 129/Sv mice carrying the same deficiency (A129), the lethal dose is higher and 100% of mice die at 10^7 pfu. However, when anti-DENV antibody is administered 24 hours prior to infection, D220 causes 100% lethality with 10^5 pfu in both A-B6 and A129 mice. The mortality induced by D220 in A-B6 and A129 mice appears due to a vascular leakage phenotype similar to that previously described with D2S10 in AG129 mice, and occurs 3.5-5 days post-infection. Further characterization of infection kinetics and phenotype is underway. Full-length sequencing of the viral genome revealed that, compared to D2S10, D220 carries four amino acid substitutions; likely most important are non-conserved substitutions at position 122 in the viral envelope (E) protein and position 228 in the non-structural (NS) 1 protein. The susceptibility of C57BL/6 mice further opens a broad range of immunological methods and genetically deficient mice that are readily available in this strain background. The development of this virus enables study of the mechanism of dengue pathogenesis, testing of antiviral compounds, and investigation of the immune response to DENV in less immunocompromised mice.

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MECHANISM OF ACTION OF THERAPEUTIC MONOCLONAL ANTIBODIES IN A DENGUE MOUSE MODEL

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Dengue hemorrhagic fever and dengue shock syndrome (DHF/DSS) are life-threatening complications following infection with one of the four serotypes of dengue (DENV). Epidemiological evidence has suggested that the greatest risk factor associated with the development of DHF/DSS is prior infection with a different serotype. Recently, we have published that antibodies alone are sufficient to enhance a sub-lethal DENV2 infection and cause lethal disease in AG129 mice, with features similar to human DHF/DSS. Subsequently, we identified a monoclonal antibody (MAb) targeting the E-domain II (DII) fusion loop, E60, which is therapeutic 24 and 48 hours following an antibody-enhanced lethal disease when aglycosylated to prevent binding with FcγR. Here we characterize a panel of nine human or mouse-human chimeric MAbs and their aglycosylated variants and correlate their therapeutic and prophylactic potency *in vivo* with various *in vitro* characteristics, including epitope specificity, mechanism of neutralization, neutralizing titer, and affinity. These nine MAbs target five different epitopes on the E protein and are moderately to strongly neutralizing *in vitro.* Initial results indicate that these aglycosylated MAbs are effective as therapeutics following a lethal infective dose of DENV. However, two of these Mabs, which target two distinct epitopes, are also completely therapeutic following an antibody-enhanced, lethal infection. Further analysis indicates that both neutralizing potency and MAb affinity correlate with antibody-enhanced therapeutic efficacy with MAbs targeting the EDII fusion loop but not with MAbs targeting different epitopes, including the EDIII C-C′ loop, EDIII A strand, or EDI/II dimer interface. These modified antibodies also provide a unique tool to study the early kinetics of a lethal DENV infection. These studies should further our understanding of ADE and the mechanism of action of therapeutic MAbs that are effective in preventing lethal disease.

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EMERGENCE OF A NEW LINEAGE OF DENGUE-2 VIRUS WITH INCREASED PATHOGENESIS IN PERU

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Dengue fever is an arboviral disease caused by four antigenically distinct but related viruses. Dengue infection occurs in more than 100 countries with an estimated 50-100 million infections annually. The clinical spectrum of dengue disease is broad: most dengue infections are inapparent or present as a debilitating but self-limiting undifferentiated febrile illness; however, a small number of cases progress to more severe forms of the disease (dengue hemorrhagic fever and dengue shock syndrome). While historically the largest burden of severe disease has fallen on Southeast Asia, there is an increasing burden of severe disease on the Americas as well. Over the past 20 years there has been an 8.3 fold increase in number of dengue hemorrhagic fever (DHF) cases in the Americas, and in late 2010/early 2011, the Amazonian city of Iquitos, Peru, experienced its largest DHF outbreak ever. The DHF outbreak in Iquitos corresponded with the introduction of a dengue-2 virus (DENV-2) belonging to the Asian/American II lineage. While DENV-2 had previously circulated in Iquitos, this was the first time strains of this lineage had circulated there. In order to better understand the increased disease severity associated with this virus, we compared replication kinetics, vectorial capacity and whole genome sequences of DENV-2 isolates collected during the recent DHF outbreak to isolates previously collected in the area. Our phylogenetic analyses based on complete genome sequences confirmed the introduction of the new lineage of Asian/American genotype. Sequence analyses revealed a high degree of conservation in the 5′- and 3′- untranslated regions, but considerable differences at the nucleotide and amino acid levels were observed within the open reading frame. Additionally, replication was compared in cultured cells, where lineage II strains produced a significantly higher output of progeny in human liver cells, but not in mosquito cells. Understanding the genetic relationships and phenotypic differences of this emergent lineage may provide valuable insight into DENV emergence and guide monitoring of future outbreaks.

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REPLICATION DYNAMICS OF DENGUE VIRUS TYPE 1 FROM TWO HAWAII OUTBREAKS IN LOCAL *AEDES ALBOPICTUS* MOSQUITOES

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Dengue virus is a globally expanding pathogen accounting for increasingly severe epidemics, whose genetic diversity is partially responsible for differential transmission and epidemic potential. Dengue is transmitted primarily by the anthropophilic mosquito *Aedes aegypti*, and occasionally, as in Hawaii where the former is rare, by the Asian tiger mosquito *Aedes albopictus*. To understand the importance of viral strain in transmission by vectors, we took advantage of the well-documented outbreak history of dengue in Hawaii and infected local mosquitoes with 4 different virus strains isolated from Hawaii as early as 1943. Mosquitoes from a wild-founded Oahu *Ae. albopictus* colony were allowed to feed on infected blood (50% human blood and 50% supernatant from viral C6/36 culture) containing one of four strains of dengue virus type 1 (DENV-1) or a negative control. The strains included three isolates from the 2001 Hawaii outbreak: two viruses linked to Tahiti where the 2001 epidemic was characterized by high transmission and severe disease, and one virus linked to an attenuated outbreak in American Samoa at the same time. The final virus was an isolate from the 1943 Hawaii outbreak, representing an earlier form of DENV-1. Up to four replicates of each treatments were fed to 4-5 day-old *Ae. albopictus* mosquitoes (colony generation F7) resulting in 1401 blood-fed females. A subset of mosquitoes were sacrificed immediately post blood meal, at 5 hours post feeding, and again at 1, 4, 7, and 14 days post-infection. At each time point, 2-4 mosquitoes per replicate were dissected into midgut, salivary glands, and remaining carcass. An additional 2-5 mosquitoes were collected whole for additional quantification of virus infection. Total RNA was extracted from each tissue type, transcribed to cDNA and quantified for DENV by qPCR for the NS5 gene. Although all virus treatments resulted in high rates of mosquito infection, dissemination to the salivary glands by day 7 were highest for one of the Tahitian derived strains, and lowest for the American Samoan derived strain and another of the Tahitian strains. The 1943 strain was intermediate. Results for the other time points will also be presented. The identification of virus strain-specific differences in mosquito infection dynamics suggests an important role for differential viral fitnesses in epidemic dynamics. Support was provided by NIH-RR018727, NIH-AI065359, NIH-RR003061, and DOD-06187000.

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CONSTRUCTION AND CHARACTERIZATION OF CHIMERIC JAPANESE ENCEPHALITIS/DENGUE VIRUS TYPE 4 VACCINE CANDIDATES

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Japanese encephalitis virus (JEV), a member of the flavivirus genus, is a leading cause of viral encephalitis worldwide and vaccination remains one of the most effective ways to prevent disease. A safe, live-attenuated vaccine would be ideal because of the potential for low cost production, lifelong immunity following a single dose, and the possibility of combination with a live-attenuated dengue vaccine. Here we describe the construction of six different chimeric JEV/dengue virus type 4 (DEN4) vaccine candidates. The chimeric viruses were generated by replacing the membrane precursor (prM) and envelope (E) structural genes of DEN4 or DEN4Δ30, which contains a 30 nucleotide deletion in the 3′-untranslated region (UTR), with those of JEV strain India/78. This strategy has successfully produced vaccine candidates for other flaviviruses, and special attention has been paid to the nature of the capsid (C)/prM cleavage junction, which contains the viral protease and furin cleavage sites, and plays an important role in virus viability. Therefore, chimeric cDNA molecules were constructed containing either a JEV, DEN4, or West Nile virus C/prM junction. All six recombinant JEV/DEN4 chimeras were recovered in C6/36 mosquito cells from transcripts produced *in vitro*, followed by terminal dilution in Vero cells to acquire biologically-cloned and Vero cell-adapted viruses. These viruses were sequenced and shown to have acquired a single NS4B gene mutation, such as P101L, T105I, L112S or V109A/A240V, which have previously been identified as being important for Vero cell adaptation of DEN4 and other DEN4 chimeric viruses. Mutations were also identified in E (F167S, M240L, V253F, Q264H, K312R, S364P, G413R, I430T, and M475V), NS2A (M168V), NS3 (S158L and R202I) and the 3′-UTR among the various subsets of the chimeric viruses, and may also be important for Vero cell adaptation. These six chimeric JEV/DEN4 vaccine candidates are currently being evaluated in mice to determine the level of neurovirulence and neuroinvasiveness compared to the wild-type JEV parent.

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HEPATITIS C VIRUS REPLICON SENSITIZES HOST CELLS TO TRAIL INDUCED APOPTOSIS BY UP-REGULATING DR4 AND DR5 THROUGH A MEK1-DEPENDENT PATHWAY

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shown that HCV infection can sensitize host cell to TRAIL-induced apoptosis, while the mechanism by which HCV regulates TRAIL pathway remains to be determined. Here we demonstrated that HCV replicon sensitized Huh7 cells to TRAIL-induced apoptosis by up-regulating two TRAIL receptors death receptor 4 (DR4) and death receptor 5 (DR5). Elimination HCV replicon from HCV replicon cells reversed the up-regulation of the expression of DR4 and DR5 and decreased the sensitivity to TRAIL. We found that HCV replicon enhanced Sp1-mediated transcription of DR5 gene and mutation of Sp1 binding sites on 5′-flanking promoter region of DR5 or knockdown of Sp1 by specific siRNA decreased expression of DR5. Furthermore, we found that PD98059, an inhibitor of MEK1, inhibited the enhancement of expression of DR4 and DR5 mediated by HCV replicon, and over-expression of MEK1 in Huh7 cells enhanced the promoter activity of both DR4 and DR5. Also we found phosphorylation of MEK1 increased and knock down of MEK1 by siRNA reversed the increased expression of DR4 and DR5 in HCV replicon cells. This finding may help to further unravel the pathogenesis of HCV and provide new therapeutic interventions of HCV infection.

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IDENTIFICATION OF NEUTRALIZING EPITOPES ON CHIKUNGUNYA VIRUS ENVELOPE PROTEIN

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In order to obtain comprehensive monoclonal antibody (MAb) epitope maps at the resolution of individual amino acids, we developed a novel technology, Shotgun Mutagenesis Epitope Mapping. This approach offers the capability of mapping both linear and conformational epitopes, even for structurally complex proteins such as oligomeric and glycosylated Envelope proteins. Integral Molecular is using this technology to generate detailed and comprehensive epitope maps of the immunodominant Envelope protein (E2/E1) of Chikungunya virus (CHIKV). A comprehensive mutation library for the CHIKV S27 strain Env protein was created in which every residue was individually mutated to a defined substitution, expressed

in human cells, and analyzed for its effect on antibody reactivity. For each MAb tested, Shotgun Mutagenesis identified amino acids on Env that are critical for antibody binding. These residues will enable generation of detailed epitope maps that can be visualized on the E2/E1 protein structure. Our goal is to map epitopes on CHIKV Env protein, determine how they contribute to neutralization of infection, and how they relate to protein function. We expect that this approach will help define the range of immunodominant structures on CHIKV Env and identify novel neutralizing antibody epitopes that can be used for therapeutics, diagnostics, and vaccine development.

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ZIKA VIRUS FROM FEVER SYNDROMIC SURVEILLANCE IN CAMBODIA

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In collaboration with the Cambodian Ministry of Health, US Naval Medical Research Unit 2, Cambodia has conducted fever syndromic surveillance study since 2006. Patients are currently being enrolled from 11 in 5 provinces in south central and northeastern Cambodia. Upon enrollment, respiratory specimens, whole blood and serum were collected. Testing was performed for viral, bacterial and parasitic pathogens at a centralized laboratory in Phnom Penh. Dengue fever is tested for by serological (IgM) and molecular methods (real time PCR). The real time PCR utilized in this study is a universal flavivirus screen (reported previously) that targets the NS5 gene. In August 2010, a 3 year-old child was enrolled with clinical complaints of fever, headache, sore throat, and cough. Serological tests for dengue from both the acute and convalescent specimens were negative. The serum was positive by the flavivirus screen but negative by dengue and Japanese Encephalitis specific PCR tests. Nucleic acid sequencing of the amplicon isolated by gel purification produced a 100bp fragment with 100% sequence identity to Zika virus. This is the first case of Zika virus identified in Cambodia.

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ISOLATION AND CHARACTERIZATION OF A TICK-BORNE ENCEPHALITIS VIRUS STRAIN FROM *IXODES PERSULCATUS* TICKS FROM MONGOLIA

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Tick-borne encephalitis virus (TBEV), a member of the family flaviviridae, causes one of the most important inflammatory didease of the central nervous system (CNS), namely severe encephalitis in Europe and Asia. In Mongolia TBE is known since the 1980s. The numbers of human cases have been increasing during the last years. Endemic areas of TBE associated with severe CNS diseases have been reported mainly in the provinces (Aimak) Selenge and Bulgan in Northern Mongolia close to the Russian border.We report the first isolation and preliminary genetic characterization of a TBE virus strain from ticks collected in Mongolia. 68 ticks (Ixodes persulcatus) were collected by flagging in the Bulgan district near Khylganatt in the North of Mongolia in July 2010. Ticks were homogenized individually and supernatants were used for nucleic acid extraction (QIAGEN Viral RNA Extraction Kit). Extracted RNA was screened for TBEV-specific sequences by real-time RT-PCR. Two out of 68 (2.9%) tested ticks RNAs were reactive. The real-time RT-PCR positive tick supernatants were inoculated into Vero cells. A TBEV strain (MucAr M14/10) could be recovered from one of the two positive tick supernatants. The second positive tick supernatant with a lower TBEV RNA content proofed negative in cell culture. By conventional RT-PCR targeting the complete genome of the TBE virus strain could be amplified. Sequence comparison of the complete genome and of particular genes with other TBE virus strains of different subtypes revealed the highest homology on the nucleotide level and on the amino acid level to three strains of a subclade of the Sibirian subtype of TBE virus, the strains Zausaev (AF527415), the strains IR99-2m7 (AB049351) and Lesopark (GU121966), respectively. The data imply that TBE virus in Mongolia was introduced only recently by anthropogenic activities like road and/or railway construction.

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IMPACT OF THE JAPANESE ENCEPHALITIS (JE) IMMUNIZATION PROGRAM WITH LIVE, ATTENUATED SA 14-14-2 JE VACCINE IN NEPAL

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Japanese encephalitis (JE) cases have been reported in Nepal since the mid-1970s. In 2006, the Ministry of Health and Population introduced an immunization program to control JE. By 2009, immunization campaigns had been conducted in 23 JE-endemic districts. Campaigns targeted children 1-15 years of age (11 districts) or the whole population ≥1 year of age (12 districts) with a single dose of live, attenuated SA 14-14-2 JE vaccine. To evaluate the impact of the program, we analyzed acute encephalitis syndrome (AES) and laboratory-confirmed JE case surveillance data collected from 2004-2009 through Nepal's routine surveillance system. Expected AES and JE incidence rates and observed post-campaign rates in each district were compared. For AES, the observed post-vaccination incidence of 7.5 per 100,000 population in the 23 districts where JE immunization campaigns were conducted was 58% (95% CI 56%-60%) lower than the expected incidence of 17.9 per 100,000 if no campaigns had occurred. The greatest impact was in the four high-risk western Terai (plain) districts where the observed incidence of 6.6 per 100,000 was 84% (95%CI 83%-85%) lower than the expected incidence of 41.5 per 100,000. For JE, the observed incidence of 1.3 per 100,000 population in the post-campaign period in the 23 districts was 72% (95%CI 69%-75%) lower than the expected incidence of 4.6 per 100,000. As with AES, the impact on JE was greatest in the four high-risk Terai districts; the observed incidence of 1.9 per 100,000 was 84% (95%CI 81%-86%) lower than the expected incidence of 11.7 per 100,000. Although this analysis was limited by availability of only short-term post-campaign data in some districts, it demonstrated that the SA 14-14-2 JE vaccination program has had a clear impact on AES and JE incidence in Nepal. As additional surveillance data are available, further analysis will provide greater accuracy in the assessment of campaign impact. An ongoing routine infant immunization program will be essential to ensure the achievements in JE control are maintained.

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DEVELOPMENT AND CHARACTERIZATION OF A NONHUMAN PRIMATE MODEL FOR EBOLA VIRUS: SEQUENTIAL SAMPLING STUDY OF EBOLA ZAIRE VIRUS IN NONHUMAN PRIMATES BY AEROSOL EXPOSURE

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Ebola virus (EBOV) is a single-stranded negative-sense RNA member of the *Filoviridae* that causes hemorrhagic fever (HF). Since its discovery in 1976, this zoonotic virus has caused epidemics with high case fatalities. Although EBOV causes sporadic outbreaks in sub-Saharan Africa, it is of significant concern from a biodefense perspective because of case reports of EBOV infections outside of Africa and because the virus may be spread on aerosols. To develop a standard animal model, we have employed aerosol exposure of rhesus macaques to Zaire Ebola virus in a sequential sampling study. The results presented herein are derived from a robust sequential sampling study (28 animals) of EBOV HF in rhesus macaques challenged by a lethal dose of aerosolized EBOV and sampled on Days 1, 3, 4, 5, 6, 7 and 8. The parameters measured include: clinical symptoms; weight and temperature; complete blood counts (CBC); blood chemistry; cytokines; viral levels; coagulation; and pathology. Clinical signs included nonresponsiveness, gastrointestinal changes (no output), diarrhea, reduced food consumption, dehydration, rash, dyspnea, weakness, and depression. CBCs demonstrated that white blood cells, lymphocytes, monocytes, and platelets decreased, whereas basophils increased. Blood chemistry measurements showed increases in blood urea nitrogen, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and creatinine. Measurements of cytokine/chemokine levels showed strong increases in hepatocyte growth factor, MIG, INF- α, IL-1RA, IL-6, IL-10, IL-15, eotaxin, MIP-1β, and MCP-1. Pathology analyses demonstrated viral staining in macrophages and dendritic cells and progression to liver, spleen, and lymph nodes in the first 3-5 days after exposure. Thereafter the virus was found in kidney, adrenal gland, thymus, bone marrow, pancreas, and gastrointestinal tract. Activation of the coagulation cascade and the production of d-dimers and fibrin deposition was prominent. To attempt to validate this model the observed disease course was compared to available data from intramuscular Zaire Ebola virus challenges and the limited data available from reports of human disease.

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IMMUNE RESPONSE INDUCED BY LIVE-ATTENUATED JAPANESE ENCEPHALITIS VACCINE (JE CV) NEUTRALIZE RECENT WILD-TYPE JAPANESE ENCEPHALITIS VIRUS (JEV) ISOLATES FROM SOUTHEAST (SE) ASIA AND INDIA

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During clinical development of JE-CV, the neutralization ability of vaccine-induced antibodies was assessed against the vaccine virus (JE-CV) and against well characterized wild-type (wt) viruses isolated between 1949-1991. We sought to assess whether JE-CV-induced antibodies can also neutralize recent wt JEV isolates, representative of currently circulating genotype 1 and 3 JEV strains. Sera from 12-18m/o children who received a single dose of JE CV in a phase III study in Thailand and the Philippines (ClinicalTrials.gov NCT00735644) were randomly selected and pooled. Pooling was based on Day28 post-vaccination neutralizing antibody titers assessed by PRNT50 to JE-CV. Eight serum pools of differing titer ranges were prepared from 7-20 samples / pool, all samples used in pooling except one were from children who were JE-naïve before vaccination. Two recent isolates were obtained from the WHO Flavivirus Diagnostics Reference Laboratory for Asia at the Center for Vaccine Development University of Mahidol, Thailand: JEV-SM1 from a mosquito in Thailand, 2003; and JEV-902/97 from a clinical case in Vietnam, 1997. A single analyst performed 3 independent PRNT50 assays against these 2 isolates, as well as against 4 JEV tested previously during the development program, including JE-CV. Results were compared using geometric mean titer and median values of the 3 independent tests. All positive titer serum pools from JE-CV-vaccinated children neutralized the 2 recent JEV isolates. Within each serum pool GMT and median titers against isolates were similar generally within one 2-fold dilution, ranging from 84-980 for the low titer-high titer pools. Neutralization titers against recent wt strains were comparable to those against previously tested JEV, including the vaccine virus. Consistent with previously generated data on the neutralization of wt JEV isolates, immune responses induced by JE-CV neutralize recently isolated virus from SE Asia and India.

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YELLOW FEVER EPIDEMIOLOGICAL SITUATION AT 2010 IN BURKINA FASO

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A resurgence of yellow fever has been noticed in Africa (1) and this situation is particularly serious in West Africa. In Burkina Faso, since 2004, there have been outbreaks of this disease. The control strategy of yellow fever recommended by WHO and applied in the country is based on four key pillars including enhanced surveillance of the disease. We intend to review the situation of the surveillance of yellow fever in 2010 at the National Reference Laboratory of Yellow Fever in Centre MURAZ of Bobo Dioulasso. The serum/plasma of patients with fever and jaundice of all the health districts of the country are received at the laboratory according to the protocol defined for the national surveillance. The samples are accompanied by a form of investigation and kept at +4°C in coolers during transport. ELISA was used to search for specific IgM yellow fever. For the year 2010, 970 samples of febrile jaundice were received from 13 health regions. 935 (96.40%) samples were adequate (N ≥ 90%). Vaccine recipients were among 401 (41%) and those not vaccinated 347 (36%). The transmission of samples was done on time (≤ 7days) for 670 (69.07%) cases. 11 aliquots have been diagnosed positive for IgM specific for yellow fever and sent to the Pasteur Institute in Dakar, 8 (0.82%) were confirmed positive and 03 (0.31%) classified doubtful. Of these 08 positive samples, 07 cases were from the same region (Cascade) and none were vaccinated. In spite of the carried out efforts, cases of yellow fever are diagnosed in Burkina Faso, especially at not vaccinated subjects. it is important in spite of the monitoring to carry out vaccination campaigns apart from the programs of response.

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INVESTIGATION OF WEST NILE VIRUS RNA IN BLOOD DONORS BY REAL-TIME RT-PCR

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West Nile Virus (WNV), a member of the family Flaviviridae, is an enveloped RNA virus. Primary reservoir hosts of this virus are birds, but the virus can cause various infections in humans and other mammals. WNV infection is generally asymptomatic, but this virus may cause a wide range of different clinical forms from mild WNV fever to neurodegenerative diseases with high mortality. The most common and natural way of transmission of WNV infections is mosquito bites, but that are shown the humans can be infected by this virus with different routes. The most

important non-mosquito transmission route is contaminated blood and blood products. WNV seropositivity has been reported in various regions of Turkey and around the province of Ankara so far. Seven patients with West Nile fever were reported in the western regions of Turkey in August 2010 and three of these patients resulted in death. In this study, we aimed to investigate the risk of WNV transmission through blood and blood products, especially for the region of Ankara in Turkey. For this purpose we included 729 serum samples in the study that are obtained from healthy volunteer blood donors. The vast majority of donors were male (97.5%) and resident in Ankara (96.3%). We investigated the presence of viral RNA in the serum samples by real-time RT-PCR. WNV RNA was not detected in serum samples. This result may be due to absence of patient with viremia among blood donors included in this study. Previous studies have shown that seroprevalence of WNV infection was 0.6 to 2.4% among blood donors in this region. For this reason, the risk of WNV infection in blood donors should not be ignored.

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WEST NILE VIRUS-INDUCED CYCLOOXYGENASE-2 PROMOTES INFLAMMATION IN THE BRAIN

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Inflammatory immune responses in brain initially triggered to clear West Nile Virus (WNV) promote blood-brain barrier (BBB) disruption, infiltration of immune cells and neuronal death. However, the mechanisms by which WNV modulates these inflammatory responses are unclear. We previously demonstrated that matrix metalloproteinases (MMPs) play an important role in WNV-induced BBB disruption. Cyclooxygenase-2 (COX-2) and its product prostaglandin E2 (PGE2) can initiate inflammation via cytokines and matrix metalloproteinases (MMPs). This study was aimed to identify and characterize the pathophysiological consequences of COX-2 expression in WNV-infected human brain cortical astrocytes (HBCA) and in mice brain. C57BL/6 mice were infected with WNV (NY99) at 10^2 PFU and COX-2/PGE2 levels were measured. Primary HBCA were infected with WNV at MOI-5 in the presence or absence of specific COX-2 inhibitor (NS398), and expression and activity profile of COX-2, cytokines and MMPs were analyzed. In mice brain, WNV infection increased the expression of COX-2 mRNA and protein at day 7 after infection, which correlated with peak virus titers. The expression of COX-2 in WNV-infected HBCA was 10- to 87-fold high from days 1 to 4 after infection, which coincided with peak expression of multiple MMPs, IL-1β, -6 and -8 and PGE2. Treatment of HBCA with NS398 decreased the expression and release of WNV-induced MMPs, IL-1β and cytokines by 60 to 88%. In conclusion, our data identifies astrocytes as one of the sources of COX-2-derived PGE2 that initiates multiple downstream pathological events such as cytokine and MMP production, thereby contributing to two major hallmarks of WNV-encephalitis, neuroinflammation and BBB disruption. The ability of COX-2 inhibitors to modulate WNV-induced COX-2 and PGE2 signaling should be further investigated in an animal model as a potential approach for the clinical management of WNVE.

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MUTATIONS IN THE PRM PROTEIN OF WNV INHIBIT SECRETION OF VLP BUT NOT VIRUS

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West Nile virus-like particle (VLP) mutants differing in amino acids (AAs) of the prM protein were produced and used to identify epitopes reactive with three human monoclonal antibodies. We discovered that four prM mutations (T20D, K31A, K31V, or K31T) reproducibly resulted in undetectable levels of VLP secretion. To determine the effects these prM mutations had on the virion, they were introduced into the West Nile virus (WNV) infectious cDNA clone. In all cases, infectious virus was recovered following transformation of C6/36 cells. Replication of the prM K31A and T20D viruses were similar to wild-type (wt) WNV in both mosquito (C6/36) and mammalian (Vero) cells, with no compensatory AA changes in either the prM or envelope (E) proteins. The prM K31T virus titer was reduced 10-fold when grown in both C6/36 cells and Vero cells as compared to wt WNV with a stable prM and E gene sequence. The prM K31V had reduced levels of replication in both Vero cells (10-fold) and C6/36 cells (100-fold). Sequencing revealed that after transfection of C6/36 cells (C=0), prM K31V incurred a compensatory mutation of prM, L33P. Our results suggest that while mutations in the prM can reduce or eliminate secretion of VLPs following transfection of COS cells, these same prM mutations have less or no effect on viral replication in both Vero and C6/36 cells. This difference may be due to the high level of prM seen in WNV VLPs grown in mammalian cells as compared to virus, or to the differences in structure and symmetry of the VLP compared to virus.

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BLACK FLY PHEROMONES AND THE MONITORING AND ERADICATION OF ONCHOCERCIASIS

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Onchocerciasis or river blindness disease is a parasitic disease caused by infection from the nematode Onchocerca volvulus. The parasite is transmitted to humans by black fly vectors of the genus Simulium. Most of the infections occur in central Africa, with significant incidence also in Central and South America. According to the World Health Organization an estimated 18 million people suffer from onchocerciasis. However, since the disease is endemic to only the poorest regions of the world it is difficult to gain exact reports and statistics about the disease. The current method for monitoring the spread employs human bait, which is neither optimal nor ethically sound. The need for a new monitoring method is very important. It was noticed that gravid flies are attracted to egg masses recently deposited by other flies of the same species. This paper will describe our efforts to isolate and identify the pheromone responsible for this attraction, which we then plan to develop as bait for a field trap for monitoring vector pressure. In the long term, field traps may be useful in eradication of the disease.

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FACTORS AFFECTING MASS DRUG ADMINISTRATION PROGRAM FOR THE ELIMINATION OF LYMPHATIC FILARIASIS IN A DISTRICT IN GHANA

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Lymphatic filariasis (LF) is the second most common vector-borne parasitic disease after malaria in many tropical countries. Worldwide, the WHO estimates more than 1.3 billion people in 81 countries are threatened and over 120 million people are currently infected. It is endemic in the northern and southern sectors in Ghana. The disease is targeted for elimination by 2020 and the key intervention is mass drug administration (MDA) using a single annual dose combination of ivermectin or DEC and albendazole for 5-6 years. The MDA has been running in Ghana since 2000. The aim of this study was to identify factors responsible for compliance and non-compliance to MDA. Ninety nine communities were stratified into 4 strata according to MDA coverage rates and 17 were randomly selected and studied. A six part pre-tested questionnaire was applied to all respondents and the key issues explored included knowledge

of MDA, reasons for compliance and non-compliance, mode of drug distribution and acceptance of MDA. Observed coverage of MDA of 65.4% in 2006 increased to 86.3% in 2008. Overall MDA acceptance was 99.7% but drug compliance was 86.0% among respondents. Some 14% of study respondents perceived the drug was good for their health. Reasons for non-compliance included travelling (14%) and view that the programme was not necessary (4%). Awareness that the drugs prevent LF was a major contributor to compliance. Door-to-door mode of drug delivery was the most preferred (82.1%). Drug compliance showed significant positive correlation with awareness of MDA. Door-to- door delivery using community volunteers was more successful than delivery from health centres. Efforts to eliminate the disease are however hampered by community ignorance, misguided education and adverse effects. High MDA acceptance could be sustained with simplified education using volunteers.

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FACTORS ASSOCIATED WITH *WUCHERERIA BANCROFTI* MICROFILAREMIA IN AN ENDEMIC AREA IN MALI

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Lymphatic filariasis (LF) due to *Wuchereria bancrofti* (Wb) is endemic in all 8 administrative regions of Mali and represents an important public health problem. Although mass drug administration (MDA) strategies have been implemented in most regions, a number of factors may influence Wb microfilarial load and thus potentially affect the efficacy of MDA at the individual or community level. These factors include spatial clustering, coinfection with *Mansonella perstans* (Mp) and bednet use. To determine the effect of these factors on Wb prevalence and microfilarial levels, cross-sectional data obtained during screening for an interventional study in Bougoudiana and Tieneguebougou, neighboring villages (<10 km apart) in the district of Kolokani, were examined. A total of 372 volunteers (235 males and 137 females) aged 14 to 65 (mean of 34 years), were questioned about bednet use and prior participation in MDA. Wb and Mp microfilarial (mf) loads were assessed by calibrated thick smear, and Wb circulating antigen (WbCAg) levels were determined using the TropBio™ ELISA. All volunteers were georeferenced for disease distribution analysis and mapping. The overall prevalence of Wb microfilaremia was 17%. Prevalence was significantly higher in Tieneguebougou than in Bougoudiana (23.2% vs. 10.7%, respectively; p=0.0015; Fisher's Exact test); however, positive and negative individuals were randomly distributed across the two villages (Moran's I spatial statistic = -0.01, Z score =0.1, P>0.05). Of the 196 subjects with detectable Mp microfilaremia, 47 (24%) had detectable Wb mf, as compared to 17/177 (9.5%) Mp-negative subjects (p<0.001, Fisher's Exact test). However, the geometric mean Wb load was comparable in the two groups (214 vs. 123 mf/ml; p=0.17, Mann-Whitney U test). Only 36% of subjects gave a history of bednet use at the time of the survey and 52% had received antifilarial therapy as part of MDA one year prior to the study. Neither a history of bednet use nor prior antifilarial therapy had an effect on prevalence of Wb microfilaremia, Wb mf load or WbCAg positivity. Thus, of the factors examined, only Mp infection had a significant influence on the prevalence of Wb microfilaremia. Whether the relationship between Mp and Wb is due to host factors or the biology of the parasites remains to be elucidated.

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LACK OF EFFECT OF FILARIAL INFECTION ON ASYMPTOMATIC MALARIA PARASITEMIA IN KOLOKANI, MALI

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Wuchereria bancrofti (Wb) and *Mansonella perstans* (Mp) are blood-borne filarial parasites that are endemic in many countries of west and central Africa, including Mali. Their geographic distribution overlaps considerably with that of malaria, and coinfection is common. Although prior studies have demonstrated effects of filarial infection on the immune response to malaria, the influence of filarial infection on asymptomatic carriage of malaria parasites is unknown. To address this question, *Plasmodium falciparum* (Pf) parasitemia was assessed monthly throughtout the transmission season in 83 asymptomatic subjects participating in a study of the effects of filariasis on clinical malaria in two villages in Kolokani, Mali. Filarial infection was defined by the presence of Wb or Mp microfilariae on calibrated thick smears performed between 10 pm and 2 am and/or by positive TropBio™ ELISA for circulating filarial antigen (CFA) in serum. There were no significant differences between the filarial-positive (FIL+) and filarial-negative (FIL-) subjects with respect to age, gender and hemoglobin status. At the beginning of the transmission season, 22/36 FIL+ subjects and 29/46 FIL- subjects had Pf parasitemia (p=NS). Geometric mean Pf parasitemia was also comparable between the two groups (179.3 vs 201.2, respectively). Although the prevalence of Pf parasitemia increased over the course of the transmission season in both FIL+ and FIL- groups, no significant differences were seen between the groups with respect to prevalence of Pf parasitemia or Pf parasite load. Thus, despite differences in immune responses to malaria parasites in the setting of filariasis, asymptomatic carriage of Pf appears to be unaffected.

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CAN SCHOOL-BASED SAMPLING BE USED IN PLACE OF COMMUNITY-BASED SAMPLING TO MEASURE CIRCULATING FILARIAL ANTIGEN FOR *WUCHERERIA BANCROFTI* IN AREAS WHERE SCHOOL ATTENDANCE IS LOW?

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Population-based surveys serve as the benchmarks for monitoring and evaluating the progress of lymphatic filariasis (LF) elimination programs. From the start of LF elimination efforts to surveys to determine if mass drug administration (MDA) can be safely discontinued, these surveys are crucial for measuring program success. Large-scale surveys conducted at the household level are expensive and time consuming; there is great interest in shifting to school-based sampling that would involve less travel for data collection teams, greater ease of planning, improved facility for systematic sampling, and an entry for integration with other NTDs whose assessments depend on sampling the community survey. The prevalence of CFA in the household survey (ICT-positive) was 0.44%, compared with 0.32% in the school survey, which corresponds to a chi-squared statistic of 0.26 (p=0.61). Both of the survey results indicate a CFA prevalence that is well below the MDA stopping threshold. Based on the results of this study we conclude that there is no statistical difference between children sampled in the schools or community. These findings also indicate the feasibility of integrating NTD programs, particularly schistosomiasis and trachoma, whoseschool-age children. When school attendance is high it follows that school-based sampling should yield similar results to household-based sampling; however, when school attendance is low

the equivalency of these sampling methods remains undetermined. The purpose of this study is to determine if school-based sampling of children yields statistically equivalent results to sampling children of the same age in the community when school attendance is <70%. To address this question, two concurrent surveys of children 6-7 years old, surveyed in the household, and 1st and 2nd graders, surveyed in the schools, were conducted in the Houndé health district in Burkina Faso, where school enrollment is 57%. All surveyed children were tested for the presence of circulating filarial antigen (CFA) using ICT cards. A total of 3145 children were sampled, 1542 from the school survey and 1603 from assessments target school-age children.

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EVALUATING PROGRESS TOWARD THE ELIMINATION OF LYMPHATIC FILARIASIS IN AMERICAN SAMOA THROUGH THE ASSESSMENT OF FILARIAL ANTIGEN AND ANTIFILARIAL ANTIBODY RESPONSES

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Significant progress has been made toward the global goal to eliminate lymphatic filariasis (LF) by 2020; however, demonstrating success will depend on careful monitoring after the implementation of mass treatment interventions. As treatment goals are met, programs face the decision of when it is appropriate to stop mass drug administration (MDA). Newly modified WHO guidelines provide a protocol for conducting transmission assessment surveys (TAS) to guide this decision. A TAS was conducted in American Samoa in February 2011. A total of 1,134 children (6-7 years old) were enrolled, representing 44.5% of the students in this age range. Of the children enrolled, 956 (84.3%) were tested by ICT for the presence of filarial antigen. Two positive children (0.2%) were found by ICT with no evidence of microfilaremia by blood smear or PCR. Since the number of positive children was below the established critical value for the TAS as performed in American Samoa, an official recommendation was made to stop MDA. After MDA is stopped, programs face a new challenge in carrying out surveillance to prevent the re-emergence of transmission. A history of LF recrudescence in American Samoa after previous MDA campaigns coupled with the efficiency of the vector (*Aedes polynesiensis*) make surveillance in this setting an issue of paramount importance. Evidence suggests that detection of antifilarial antibodies provides the earliest indicator of filarial exposure. Therefore, monitoring filarial exposure through the assessment of antifilarial antibody responses may provide a useful tool for detecting potential recrudescence. During the TAS, blood was collected on filter paper, dried and stored for testing. Using a newly developed multiplex platform which allows for the analysis of multiple antigens at one time, all of these samples will be tested with three available filarial antigens (Bm14, Bm33, Wb123). Results from this study will establish a baseline for surveillance and potentially provide insight into filarial exposure in an area that has been recommended to stop MDA.

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ONCHOCERCIASIS TRANSMISSION CONTINUES IN NYAGAK-BONDO FOCUS OF NORTHWESTERN UGANDA AFTER 18 YEARS OF ANNUAL DISTRIBUTION OF IVERMECTIN

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A single dose of ivermectin through community-directed treatment with ivermectin (CDTI) was recently estimated to eliminate onchocerciasis transmission in 15 to 17 years, allowing safe withdrawal of mass drug administration programs. In Nyagak-Bondo focus of northwestern Uganda annual mass treatment has been provided for 18 years. The objective was to determine whether annual treatment could be withdrawn without a possibility of recrudescence in Nyagak-Bondo focus. Baseline skin snip microfilariae (mf) and nodule prevalence data from 1993 from 6 communities in the Nyagak-Bondo focus were compared with data collected during 2011 follow up study in 7 communities from the same transmission zone. Three hundred adults were snipped at baseline in 1993, and 180 had been assessed for nodules. In 2011, 607 adults were examined for mf and nodules. From the same communities, mf baseline data from 58 children were compared with 2011 data from 145 children aged under 10 years. All communities in the transmission zone have been receiving regular annual mass treatment, and at an annual coverage of more than 85% of eligible population. Overall, mf prevalence among adults dropped from 83.1% (with community prevalences ranging from 90% to 100%) to 23.6% (range 3.4% to 40%, $p<0.0001$). Nodule prevalence dropped from 97% (range 96% to 100%) to10.9% (range, 2.3% to 20%, $p<0.0001$). In children mf prevalence decreased from 78.5% (36.4 to 100%) to 11.9% (0 to 36.8%), $p<0.0001$). Despite a dramatic decrease in onchocerciasis infection parameters in the Nyagak-Bondo focus after 18 years of annual treatment, 2011 infection rates in adults and children are too high to consider it feasible to halt ivermectin treatment at this time without risk of recrudescence.

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LONG LASTING INSECTICIDAL NETS (LLIN) ALONE APPEAR TO INTERRUPT TRANSMISSION OF LYMPHATIC FILARIASIS IN SOUTHEAST NIGERIA

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In West Africa Lymphatic filariasis (LF) is caused by the parasite *Wuchereria bancrofti;* in rural areas LF is transmitted by *Anopheles* mosquitoes. In southeast Nigeria, which includes Imo and Ebonyi states, potential coinfection with *Loa loa* parasites prevents use of the mass drug administration (MDA) strategy for LF elimination. The Carter Center is working with the ministries of health of those states to determine if mosquito vector control for malaria by means of LLIN will impact transmission of LF. In two study areas (Abakaliki and Ohaji Egbema local governments) baseline LF antigenemia in sentinel sites averaged 29%. From April-May 2008, 139,080 LLIN were distributed to reach all age groups in the two local government areas with an additional 32,600 nets in June 2008. Household (HH) cluster surveys showed that the proportion of HH with at least one LLIN increased from 3.3% in 2007 to 92.0% immediately after distribution in 2008, with an average of two nets within HHs owning at least one net. In six sentinel villages (three in each LGA)

mosquitoes have been collected by pyrethrum knockdown every month in one room of each of 30 HH since June 2007. Collected mosquitoes were immediately dissected to determine rates of LF infection (L1-L3 stage larvae). Eighty-three percent of the collections were *Anopheles* species (*An. gambiae* sl 75 percent and *An. funestus* 7 percent). We compared mosquito collection numbers and infection rates before/around LLIN distribution for the 12 month period June 2007-May 2008 with a 14 month period starting one year after LLIN distribution (June 2008-July 2009). Mosquito captures show a trend suggesting a decrease in mosquito abundance (decreasing from 5,098 to 1,395) and infection: one infection (L1) was found in the year after LLIN were distributed compared to 38 for the year before (Chi square 7, p<.01). No L3 have been detected since LLIN were distributed compared to 11 at baseline (P=NS). The study continues, but these early results suggest interruption of LF transmission can occur with LLIN alone, without accompanying MDA.

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PREVALENCE OF *WUCHERERIA BANCROFTI* INFECTION IN AMERICAN SAMOA AFTER SEVEN YEARS OF MASS DRUG ADMINISTRATION WITH DIETHYLCARBAMAZINE AND ALBENDAZOLE

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As part of the Pacific Program to Eliminate Lymphatic Filariasis (LF), the US Territory of American Samoa initiated mass drug administration (MDA) with diethylcarbamazine and albendazole in 2000 after a prevalence survey indicated 16.5% of residents were infected with *Wuchereria bancrofti*. Monitoring in sentinel sites indicated decreasing prevalence only after the fourth round of MDA when program strategies were modified to improve drug coverage. We conducted a population based prevalence survey in 2007 to determine the impact of seven annual rounds of MDA. Using geographical data, we took a simple random sample of households from all residential building structures on the island groups of Tutuila and Manua. All residents of selected households older than 2 years of age were eligible for assessment of circulating filarial antigen. Persons testing antigen positive were examined for microfilaria (mf). Overall 1,881 out of 2,216 registered, eligible residents were examined from 394 households. The prevalence of antigenemia among all ages 2 years and above was 2.26% (upper 95%CI 2.82%). We were unable to obtain an additional blood sample on 6/43 antigen positive individuals, but microfilaremia was detected in 5 of the remaining 37 antigen positive persons. Assuming those missed were mf positive and those antigen negative were mf negative, microfilaremia prevalence was 0.6% (upper 95%CI 0.89%). Among tested individuals age-eligible for participating in all 7 rounds of MDA, the mean reported number of times taking LF drugs was 4.02 (SE 0.08). Antigenemia was associated with increasing age (p<0.001) and reported noncompliance in MDA (p=0.042). After seven rounds of MDA, of which coverage of greater than 60% of the total population was achieved in only four, antigenemia has reduced from the baseline estimate. However, transmission may have not yet been interrupted. Targeted MDA or other alternative strategies should be considered.

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UPDATE ON THE ONCHOCERCIASIS ELIMINATION PROGRAM FOR THE AMERICAS (OEPA)

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Onchocerciasis in the Americas affects six countries (Brazil, Colombia, Ecuador, Guatemala, Mexico, and Venezuela) where it was originally endemic in 13 foci. OEPA is a regional initiative operating under PAHO Directing Council resolution CD48.R12 that calls for elimination by 2012 of new ocular morbidity attributable to onchocerciasis, and interruption of transmission. The OEPA partnership includes the endemic countries, The Carter Center, PAHO, the Gates Foundation, Lions Clubs, CDC, several universities, and the Mectizan[R] Donation Program. The strategy is ivermectin mass drug administration (MDA) at least twice each year to all endemic communities, reaching > 85% coverage. In 2010, 7 of the original 13 foci had stopped their MDA programs. As a result, the total number of ivermectin treatments administered in the Americas decreased by 28% from a peak of 852,721 in 2006 (when all 13 foci were under treatment) to 616,360 in 2010. Epidemiological indicators in 2010 showed that transmission was now interrupted in the Northcentral focus of Venezuela, and the Venezuelan Ministry of Health agreed with an OEPA recommendation to stop MDA there in 2011. As a result, only 5 foci out of 13 remain under MDA (2 in Venezuela, and 1 in Brazil, Mexico and Guatemala). MDA has ceased in Ecuador and Colombia, and may cease in 2012 in Mexico and Guatemala. WHO guidelines recommend that foci removed from MDA should conduct post-treatment surveillance for a minimum of 3 years before declaring transmission 'eliminated.' In 2010, for the first time since the start of the initiative, 3 foci (2 in Guatemala and 1 in Mexico) qualified for this "transmission eliminated" category. Certification of elimination, which can only be considered by WHO when requested for an entire country, could be requested by Colombia in 2012, followed by Ecuador in 2013. Brazil and Venezuela have all the remaining eye disease and the most active onchocerciasis transmission in the region. The difficult to access endemic area shared by these two countries on their frontiers in the Amazon region is the greatest hurdle to completing onchocerciasis elimination from the Americas.

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CAN MALARIA VECTOR CONTROL IMPACT FILARIASIS TRANSMISSION IN SUB-SAHARAN AFRICA?

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The Global Programme to Eliminate Lymphatic Filariasis (GPELF) was launched in 2000 and nearly all 42 endemic countries in the Americas, Eastern Mediterranean and Asia-Pacific regions have now initiated the WHO recommended mass drug administration (MDA) campaign to interrupt transmission of the parasite. However, nearly 50% of the LF endemic countries in Africa are yet to implement the GPELF MDA strategy, which does not include vector control. Nevertheless, the dramatic scale up in usage of insecticide treated /long lasting nets (ITNs/LLINs) and indoor residual spraying (IRS) for malaria in these African countries may significantly impact LF transmission because the parasite is transmitted mainly by *Anopheles* mosquitoes. Therefore, this study aimed to examine the magnitude and geographical extent of vector control activities in the 16 African countries yet to start MDA. National data on mosquito nets, ITNs/LLINs and IRS were obtained from published literature, national reports, surveys and datasets from public sources such as Demographic Health Surveys, Malaria Indicator Surveys, Multiple Indicator Cluster Surveys, Malaria Report, Roll Back Malaria and President's Malaria Initiative websites. The type, number and distribution of interventions were

summarised and mapped at sub-national level, and compared with known or potential LF distributions. These analyses found that vector control activities had increased significantly since 2005, with a three-fold increase in ITN ownership and IRS coverage overall. However, coverage varied dramatically across the 16 countries, and some regions reported >70% ITNs ownership and regular IRS activity, while others had no coverage in remote rural populations where LF was endemic. Although these African countries are behind in initiating MDA, and populations remain at risk, the continued global financial support, and expansion of vector control activities is promising. It is not beyond the scope of GPELF in reaching its target of global elimination by 2020, however, monitoring and evaluating the impact of these activities over the next decade will be critical to its success.

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MAPPING LYMPHATIC FILARIASIS DRUG COVERAGE AND CLINICAL CASES IN MALAWI

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In 2010, Malawi completed its second year of national mass drug administration (MDA) for the elimination of lymphatic filariasis (LF). The district health centres play a key role in the distribution of drugs, and as part of the National LF Programme, have started to collate data on the local population at risk, population treated and number of clinical cases associated with LF infection i.e. lymphodema and hydrocele, into a database. The aim of this study was to map MDA coverage and clinical cases at health centre level within endemic districts to identify high risk and vulnerable populations. Data from 10 districts with medium to low levels of endemicity were available for analyses, and the location (latitude and longitude) of each health centre was geo-referenced. The epidemiological drug coverage rates (population treated/total population at risk), number of lymphodema and hydrocele cases, and prevalence rates (%) were quantified and mapped using statistical and mapping software. Analyses found that the majority of health centres had adequate epidemiological coverage rates of >65%, and those with lower coverage were dispersed or in close proximity (i.e. 10km) to those with high coverage. However, one district had several health centres with very low drug coverage <50%, which were geographically clustered in one region. The reported number of lymphodema and hydrocele cases differed between the 10 districts and totals ranged from 30 to 91, and from 127 to 340, respectively. Not all health centres reported clinical cases, however, those that did reported between 1 and 22 lymphodema, and between 1 and 152 hydrocele cases, with generally low prevalence rates of $\leq$ 2%. This study shows that developing a geo-referenced database and district maps on MDA coverage and clinical cases can help to identify and monitor health centres with low drug coverage and high morbidity levels. This will enable interventions to be targeted appropriately and improve the prospects of LF elimination in Malawi.

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EFFECT OF HERPETIC CO-INFECTIONS IN CHILDREN WITH AIDS TREATED WITH HAART

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The objective of this study was to assess herpetic co-infection in Cambodian children whit AIDS in terms of risk factors and outcome. Two groups of children whit AIDS were retrospective analyzed with univariante (Chi-square, Fisher's tests and t-test) and multivariante analysis. A logistic regression was done to identify risk factors and factors influencing outcome. Statistical analysis was performed with the open source statistical package R. A *P*-value of <0.05 was considered statistically significant. From all of 75 children with AIDS 48 had herpes coinfection (7 herpes simplex, 17 herpes zoster, 28 chickenpox). Herpes co-infection was not observed in 29 children on HAART. In univariante analysis immune reconstruction syndrome (IRS) (OR=7,33; $CI_{95\%}$ =1,76 -35,22; *P*=0,003) and otitis media (OR=2,83; $CI_{95\%}$=0,99-8,24; *P*=0,05) were significant more frequently observed among herpes-coinfected AIDS children. IRS were significant in multivariante analysis too (*P*=0,007). If herpes infection during HAART occurs, those children are of increasing risk of IRS, otitis media and can develop adverse outcome during antiretroviral therapy. Therefore vaccination against varicella and prophylaxis use of antivirals (eg. Acyklovir) in close contacts whit herpes infected children is advisable.

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BREASTFEEDING AND VITAMIN D IN COMPARISON WITH OCCURRENCE OF INFECTIOUS EVENTS AMONG HIV EXPOSED CHILDREN IN RURAL UGANDA

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A lot of new infections due to the human immunodeficiency virus (HIV) in children were acquired through mother-to-child transmission (MTCT) of HIV. Presented study is prospective study with characteristics of quantitative and confirmative scientific approach. The major view forms formative research with focus of PMTCT program and access to the feeding of HIV exposed children (HIV+/ HIV-) relating to occurrence of infectious diseases with contemporaneous administration of vitamin D in rural areas of Uganda. The research surveyed on the definition of spread feeding approaches and described local performance associated with choice of feeding and the purpose in view was associated with impact on breastfeeding, HIV status and administration of vitamin D supplementation and occurrence of infectious event. We found, that breastfeeding in whole group of HIV exposed children under 3 months is low and prevalence of infections is high. Analysis by the event of diarrhea or pneumonia among HIV exposed children relating to breastfeeding and administration of vitamin D showed significant correlation in occurrence of diarrhea among HIV positive children 59% vs. 30,43% among HIV negative group (*P* = 0,02). Absence of HIV infection, breastfeeding and administration of vitamin D were associated with statistical significant decreasing of infectious event (*P* < 0,001). In this study was find significant impact upon infection presentation when using daily vitamin D supplementation in both children HIV negative even HIV positive. We suppose that these observation can be important for further research among HIV infected children with focusing on deeper understanding of action of vitamin D and HIV infection in consideration of prevention of infectious events among children living with HIV.

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ROLE OF MRSA AND ESBL-PRODUCING ENTEROBACTERIACEAE DECREASED DURING HAART: SEVEN YEARS FOLLOW UP IN CHILDREN WITH AIDS

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The aim of this study was to assess prevalence of resistant gram-positive organisms (MRSA, PRP) and multiresistant gram-negative bacteria (ESBL-producing *Klebsiella* spp., *Serratia* spp, *Enterobacter* spp, MDR-*Acinetobacter*, *Pseudomonas aeruginosa*) among respiratory isolates from Cambodian children with AIDS within 7 years follow up. All children with AIDS on HAART were screened every 6 month within 7 years for respiratory isolates of drug-resistant bacteria. Of 116 children 408 isolates were detected and tested for antibiotic resistance. We detected 206 gram-positive and 202 gram-negative: MRSA 47%, PRP 14%, ERY-R S. pyogenes 8%, ESBL Klepsiella spp. 10,5 %, ESBL plus Enterobacteriaceae spp., MDR-R *P. aeruginosa* 10 %, MDR-R *A. baumani* 8%. The proportion of MDR-R and MRSA decreased from 82% at the baseline to 33% after 7 years of HAART. HAART improve the immune response increasing the CD_4 absolute count and clearance of multiresistant gram negative bacteria and MRSA from respiratory tract of Cambodian children. ATB resistance during the 7 years follow up decreased despite the amount antibiotics for the treatment of opportunistic infections increased.

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ROLE OF HIGH SCHOOL STUDENTS' HEALTH LITERACY IN THE CONTROL OF THE HIV/AIDS EPIDEMIC IN SOUTH AFRICA

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South Africa's severe HIV/AIDS epidemic requires a health literate population to reduce the spread of infection, promote early screening and adherence to treatment. HIV counseling and testing, offered at no charge at health facilities is a critical intervention, but the uptake is low and targeting high school students is a feasible option to reach large numbers of youth in order to promote safe sexual practices. AIM. This study investigated the association between health literacy, HIV testing and sexual behaviour amongst rural and urban high school students in KwaZulu-Natal. In a cross sectional study, students (n=1076) at 10 KwaZulu-Natal public high schools completed a structured self-report anonymous questionnaire using the I-Change Behavioural Change Model as the theoretical framework, to measure awareness and motivating factors. Male students, mean age 17.08 years (SD 1.64) were older than females 16.47 years (SD 1.56) (P<0.005), but of 16.7% students who had tested for HIV, 127 were females(70.6%) and 53 males (29.4%) (P<0.005). More females than males thus supported HIV testing (P=0.002), reported self-efficacy to test (P=0.01) and intentions to test (P<0.005). In the model knowledge about HIV transmission (P=0.04), attitudes to HIV infected persons (P=0.03), perceptions of risk (P=0.01), and self-efficacy to be treated for HIV (P=0.007) predicted testing for HIV. Factors influencing sexual behaviour: Students who had used a condom at last sex were older (P=0.002), knew about HIV prevention (P=0.04) and were more positive about testing for HIV (P=0.03). Knowledge about HIV prevention and self-efficacy to test for HIV were also associated with sexual abstinence (P=0.048). Multiple partners decreased students' intentions to test (P=0.008). In conclusion, South Africa is placing renewed emphasis on HIV counseling and testing and these findings about health literacy awareness and motivating factors can assist in developing focused health promotion programmes which are gender and context specific.

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PERCEPTIONS AMONGST HIV-POSITIVE PEOPLE OF TAKING ANTIMALARIAL MEDICATION CONCOMITANTLY WITH ANTI-RETROVIRAL THERAPY: FINDINGS FROM A QUALITATIVE STUDY

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The double burden of malaria and HIV co-infection is faced by millions of people in sub-Saharan Africa. However, little research has addressed how affected individuals cope with prevention and treatment of these two diseases together. The pharmacokinetic safety and effectiveness of taking artemisinin-based combination therapy (ACT) for malaria concomitantly with anti-retroviral therapy (ART) for HIV is currently being examined through a clinical observational study in Muheza, Tanzania. We designed a qualitative study alongside the clinical trial to explore how affected individuals conceptualise co-infection and the prevention and treatment of malaria when HIV positive and taking ART. We are carrying out focus group discussions with HIV-positive people on ART as well as with HIV-negative people, for comparison. Findings will be triangulated with in-depth interviews held with key health workers delivering care to HIV-positive people in Muheza. Data collection will be completed in July 2011, and data will be analysed using an iterative, line-by-line approach based on the principles of grounded theory. Preliminary findings suggest that people hold a wide range of perceptions and experiences of taking concomitant treatments, and a variety of sources shape beliefs of danger or effectiveness of taking antimalarial medicines alongside ARVs, including religious or spiritual beliefs and information received from health workers. As data collection and analysis proceeds, these findings will further be situated in the local milieu of care and treatment. As such, we believe this study will help to inform public health interventions that aim to minimize the risks related to co-infection and co treatment of HIV and malaria.

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ACCEPTABLE OUTCOMES IN HIV/TB CO-INFECTED PATIENTS IN HAITI WITH CD4 COUNTS > 350 CELLS/MM^3 NOT STARTED ON ANTIRETROVIRAL THERAPY

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Ninety percent of the 10 million HIV/TB co-infected persons live in low- and middle-income countries, where there is often little infrastructure to regularly monitor long-term side effects associated with antiretroviral therapy (ART) and little availability of second-line treatment if antiretroviral resistance develops. Although there is clear evidence supporting the early initiation of ART in HIV/TB co-infected persons with CD4 counts less than 350 cells/mm^3, no studies to date have determined optimal timing for ART in HIV/TB co-infected patients with CD4 counts greater than 350 cells/mm^3. Partners In Health (PIH) runs comprehensive healthcare programs in support of the ministry of health in Artibonite, Haiti. Using PIH protocol, patients with HIV/TB co-infection and CD4 counts ≥ 350 are treated for TB and followed monthly. ART is started if CD4 count drops < 350 or if clinical symptoms deteriorate. In this retrospective study we reviewed medical records between, January 1, 2008 and January 1, 2011 for patients with CD4 counts greater than 350 cells/mm^3 not started on ART prior to or during tuberculosis treatment. Demographic data including

age, sex, weight, CD4 cell count at time of TB diagnosis, and ART status, as well as outcome measures of TB treatment success (cured or judged clinical improved by the health care provider overseeing their care), death, weight change, and days from initiation of TB treatment to initiation of ART were analyzed. Results are presented here of the first 20 patients. 85% were treated successfully; 90% were known to have survived until last follow-up date (a median 13.2 months from TB diagnosis). Average weight gain during TB treatment of 4.9kg was significant from no change ($p = 0.0034$). ART was started at a median 8.1 months from TB diagnosis in 35% of patients. Results from this pilot study suggest that outcomes for patients with HIV/TB co-infection and high CD4 counts in rural Haiti are acceptable in short term when patients are regularly followed in a comprehensive care clinic.

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LOW MAGNITUDE AND FREQUENCY OF HSV-2-SPECIFIC INTERFERON-Γ -PRODUCING CD4+ AND CD8+ T CELL RESPONSES DETECTED IN HIV-1 HETEROSEXUAL DISCORDANT COUPLES

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Herpes simplex virus type 2 (HSV-2), the most frequent cause of genital ulcer disease (GUD), has been shown to play a more important role than any other sexually transmitted infections (STIs) in driving HIV prevalence in Africa. In turn, HIV-1 infection leads to more frequent HSV-2 reactivations and shedding. The exact immune mechanisms involved in this virological negative immuno-synergy are unknown. In the present study we sought to assess whether HIV co-infection would affects HSV-specific T cell immunity. Nineteen HSV peptides, derived from HSV-2 glycoproteins gB and gD, were used to analyze the frequency and the magnitude of HSV-2-specific IFN-γ-producing $CD4^+$ and $CD8^+$ T cell responses in 30 HSV-2 seropositive patients and 17 HSV-2 seronegative individuals in a cohort of heterosexual Senegalese HIV-discordant couples, using ELISpot assay. The magnitude and frequency HSV-2-specific T cell responses was compared between 21 HSV-2 co-infected with HIV-1 and 9 HSV-2 mono-infected individuals. A significantly higher magnitude of IFN-γ-producing T cell responses were observed in HSV-2 infected patients compared to seronegative individuals (median, 61 vs. 0 spots/10^6 PBMC, $P = 0.001$). Moreover, twenty-four (80%) out of 30 HSV-2 seropositive patients showed significant HSV-2-specific IFN-γ-producing T cell responses compared with only 6 (35%) out of 17 HSV-2 negative subjects ($P < 0.001$). The HSV-2 mono-infected patients showed significantly higher magnitude of HSV-2-specific T cell responses compared to HSV/HIV co-infected patients (median, 140 vs. 42 spots/10^6 PBMC, $P = 0.024$). Our finding suggest that co-infection with HIV-1 in HSV-2-infected patients might be associated with reduced HSV-2 cellular immune responses. However, the interaction between HIV and HSV-2 appears complex, and precise longitudinal studies will be required to dissect their exact temporal relationship.

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HIV PREVALENCE IN RURAL SIERRA LEONE

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HIV prevalence is unknown in rural areas of Sierra Leone. HIV infection rates in Sierra Leone have an urban prevalence of approximately 20%. Intervention strategies can be implemented once this information is discovered. The purpose of this study is to document the prevalence of HIV infection in a rural area of Sierra Leone. Adult and Pediatric Patients presenting for medical care were randomly selected and underwent ELISA blood testing for the presence of HIV. Positive test results were sequentially screened with a Western Blot analysis to confirm true positives. 500 Adult patients and 100 pediatric patients were tested for HIV with 1 (0.2%) adult testing positive and 499 (99.8%) negative adult results obtained. 100 pediatric patients tested negative. Total population of patients treated was 1143. Total population of the chiefdom estimated at 2200. We were only able to screen 600 (27.3%) patients out of 2200, thus leaving a large section of the community untested. HIV prevalence is significantly less that in others areas of Sub-Sahran Africa. This presents a significant opportunity for primary prevention, intervention, and education to keep this devastating disease out of this area.

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SAQUINAVIR INHIBITS *PF*CRT-MEDIATED CHLOROQUINE TRANSPORT

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Antiretroviral protease inhibitors (APIs) such as ritonavir and saquinavir can directly inhibit the growth and development of HIV and malaria parasites. Data describing the antiplasmodial activity of APIs with many of the current antimalarial agents is still lacking and in the case of the artemisinin derivatives conflicting. However, studies with mefloquine and chloroquine (CQ) demonstrate that APIs act synergistically against *Plasmodium falciparum in vitro*. The activity of API/CQ combinations, however, appears to be related to the CQ sensitivity of parasites and the API under investigation. The rationale for these observations is not completely understood but is likely to be the result of a number of interplaying factors relating to the antimalarial action of each drug and the CQ resistance mechanisms employed by different parasite strains. The major determinant of CQ resistance in *P. falciparum* is the 'CQ resistance transporter' (PfCRT). PfCRT is an integral membrane protein located at the parasite's digestive vacuole. Its normal physiological function is unknown. However, specific mutations in this protein permit it to transport CQ away from its site of action within the digestive vacuole. As APIs are well known for their ability to inhibit proteins of the drug/metabolite transporter super-family, of which PfCRT is a member, inhibition of PfCRT-mediated CQ transport in resistant parasites may be associated with the synergy seen with these drugs against *P. falciparum*. In order to gain insights into the complex interplay of interactions occurring in CQ-resistant *P. falciparum* parasites treated with APIs we have used previously described transgenic parasites lines $C4^{Dd2}$ $C6^{7G8}$and $C2^{GC03}$, to examine the role of the CQ resistant PfCRT alleles in CQ/API interactions. We have also determined the effect of saquinavir, ritonavir and lopinavir on CQ accumulation in these parasites and in the *Xenopus laevis* oocyte PfCRT expression system. Our data demonstrate that the synergistic antiplasmodial action of saquinavir in combination with chloroquine against *P. falciparum in vitro* is dependent on PfCRT and that this antiretroviral protease inhibitor inhibits chloroquine transport mediated by the Dd2 chloroquine resistance-conferring form of PfCRT.

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BREASTFEEDING, HIV TRANSMISSION: CURRENT KNOWLEDGE AND GUIDELINES; REALITIES, CHALLENGES AND ETHICAL DILEMMAS IN AN HIV HIGH PREVALENCE AND RESOURCE LIMITED SETTING (RLS)

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Breastfeeding has been a universal and major determinant of child survival even before the HIV era. Recognition of HIV transmission through breast milk complicates promotion of breastfeeding (BF) especially in RLS where level of literacy and information dissemination is poor. International Guidelines regarding Infant feeding options and HIV in RLS have been changing leading to confusion and debate which needs to be well informed through current evidence from these settings. We describe realities, challenges and ethical dilemmas regarding infant feeding with reference to guidelines within a high HIV prevalence RLS country. Guidelines are distilled to focus on 'HIV-free survival' of the child as a primary outcome that is effectively counting an HIV infection as equivalent to a death, viewing only fatal outcomes in uninfected children as equivalent to an HIV infection. This approach ignores broader issues of nutritional status, community perceptions, maternal health status before conception, during pregnancy and after birth; growth and development of infected and uninfected children, their morbidity and mortality. Primary causes of infant deaths in RLS are; Infectious diseases, malnutrition and not being breastfed. Infant risk of becoming infected through breast milk is lower than risk of dying from other causes of not BF. On the other hand breast milk of an HIV infected mother is labeled as poison especially in RLS. Guidelines are informed by research contacted within ideal settings. Questions are raised regarding the appropriateness and implementation of these guidelines for both health care providers and mothers and communities in RLS. In Zimbabwe exclusive breastfeeding (EBF) up to 6 months has dropped from around 25% in 2005 to 6% in 2010 and the main reason is malnutrition among nursing mothers regardless of HIV status, whilst one in 3 under five children in Zimbabwe are malnourished. Realities and challenges of feeding options in the HIV era should be assessed and evaluated in relation to maternal and child nutrition and PMTCT coverage in RLS.

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STATE OF MALARIA DIAGNOSTIC TESTING AT CLINICAL LABORATORIES IN THE UNITED STATES, 2010

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The diagnosis of malaria can be a difficult undertaking in non-endemic areas such as the United States, where delays in diagnosis and errors in treatment occur too often. A nationwide survey of laboratories in the United States and its nine dependent territories was conducted in 2010 to determine factors that may contribute to diagnostic shortcomings. This survey explored the availability of malaria diagnostic tests, techniques used, and reporting practices. The survey was completed by 201 participants. Ninety percent of all respondents reported having at least one malaria diagnostic test available on-site in their laboratories. Nearly all laboratories performed thick and thin smears on-site; only 17% had access to rapid diagnostic tests on-site; and about 50% had access to molecular testing. Seventy-three percent reported fewer than five confirmed cases of malaria in their laboratory during the 12-month period preceding the survey. Twenty-eight percent stated that results of species identification took more than 24 hours to report. Only nine of 149 laboratories who performed testing 24 hours, 7 days a week complied with all of the Clinical and Laboratory Standards Institute (CLSI) guidelines for analysis and reporting of results. Though malaria diagnostic testing services were available to a majority of U.S. laboratories surveyed, very few were in complete compliance with all of the CLSI guidelines for analysis and reporting of results, and most laboratories reported seeing very few cases of malaria annually. The difficulty in adhering to the rigorous guidelines and lack of practice and proficiency may account for delays and errors in diagnosis. It is recommended that laboratories that infrequently process samples for malaria seek opportunities for practice and proficiency training annually, and take advantage of resources available to assist in species identification.

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MONITORING MALARIA PARASITE DYNAMICS IN VOLUNTEERS ENROLLED IN A MALARIA VACCINE TRIAL: A NEW TARGET PRODUCT PROFILE FOR RT-QPCR AND PFHRP-2

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Parasite biomarkers, PfHRP2/pLDH and PCR could potentially offer better diagnostic capability for malaria than microscopy. In this study, we report on malaria parasite dynamics for 112 days of passive follow-up as evaluated by microscopy, PfHRP-2, pLDH and PCR. We also report on comparison of the assays in predicting onset of clinical malaria. Blood samples were obtained from 30 adult research subjects enrolled in the FMP-10 blood stage malaria vaccine trial that was conducted between December 2008 and June 2009 at the KEMRI/Walter Reed Project Clinical Trial Center in Kombewa, Kisumu District, Kenya. Samples were obtained weekly first for the first three weeks and fortnightly thereafter during scheduled visits for a period spanning 112 days and analyzed after the end of observation period. Volunteers were asked to report for treatment whenever they fell sick. Presence of malaria was evaluated by microscopy, ELISA for PfHRP2 and pLDH antigens and RT-qPCR. Kaplan-Meier was used to evaluate proportions of study participants predicated to have parasitemia and clinical malaria by each assay at each scheduled visit. Survival proportions were highest when estimated by microscopy and pLDH and lowest by PfHRP-2 and RT-qPCR. Log rank (Mantel-Cox) test showed significant differences in the trend curves ($P<0.0001$). Of the 12 participants who developed clinical malaria, RT-qPCR/PfHRP2 correctly predicted 90% (11/12) while pLDH and Microscopy could only predict 50% (6/12). During the 112 days follow up, microscopy and pLDH ELISA detected 40 and 50 malaria events respectively, while RT-qPCR and HRP-2 ELISA detected 118 and 110 events respectively. Because of enhanced sensitivity of RT-qPCR and PfHRP-2 over conventional methodologies, there is need to re-define their target product profile from being diagnostic for purposes of disease management to monitoring parasite dynamics in the context of drug/vaccine clinical trials.

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COMPARISON OF THREE METHODS FOR THE DETECTION AND SPECIATION OF *PLASMODIUM* SPECIES IN CHILDREN AND PREGNANT WOMEN IN BANGOLAN, NORTHWEST REGION OF CAMEROON

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Effective treatment of malaria requires accurate laboratory diagnosis. Microscopy still remains the gold standard for the diagnosis of malaria. Rapid diagnostic tests (RDTs) and PCR assays are alternatives to microscopy and have been shown to be sensitive and specific. However, very few comparative studies have been reported on the three diagnostic methods in vulnerable groups. The sensitivity and specificity of microscopy, RDTs (SD Bioline kits; Pf/pan and Pf specfic kit) and PCR was used for detection and speciation of *Plasmodium falciparum* (Pf), *P. malariae* (Pm) and *P. ovale* (Po) in patients in Bangolan. A total of 54 children and 16 pregnant women were recruited for the study after obtaining an informed consent. Blood collected was used for thin and thick smears for microscopy, RDTs and blood spot on filter paper for DNA extraction and conventional PCR. Of the 70 patients diagnosed, parasitemia ranged from 520 -149600/µl. A total of 87.14% were positive by microscopy, 85.71% by RDTs and 90% by PCR. The distribution of *Plasmodium* species in the study population as identified by PCR was 72.86% Pf/Pm, 11.43% Pf/Pm/Po and 5.43%, Pm while 10% were negative. All the children were positive for malaria by microscopy though it could not clearly differentiate the various species. Of the 54 children, 94.44% tested positive with RDTs while 98.15% were positive with PCR. In pregnant women, the detection/speciation of malaria parasites was 62.5% by PCR, 50% by RDTs and 43.75% by microscopy. The Cohen's Kappa agreement between PCR and RDTs was K= 0.75 (CI = 0.28-1.22) whereas that for PCR and microscopy was K= 0.64 (CI= 0.18-1.10). PCR still remains the most specific and sensitive method. RDTs could be used for routine diagnosis of malaria in vulnerable groups as they have indicated a good concordance with PCR. Malaria infection in Bangolan is mostly due to mix infection predominantly *P. falciparum/P. malariae* and this could influence treatment outcome.

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SEVERE MALARIA AND CHILD NON-SICKLE CELL ANEMIA IN A PEDIATRIC EMERGENCY UNIT AT BONZOLA HOSPITAL: A PROSPECTIVE STUDY

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In order to adequately improve malaria survival in the different areas of Sub-Saharan Africa, there is a need to periodically generate contextual indicators on prevailing malaria case management. We explore cases and correlates (age, gender, onset of complaints, timing of admission, blood type, blood transfusion and survival/death) of severe malaria in Mbuji Mayi by reviewing medical records of all 0-5 year old children seen at Bonzola Hospital from May to July 2009. To be included, a child had to have a documented positive malaria diagnosis. To be considered severe, a malaria case had to show clinical signs of fever, diarrhea, throwing up, and asthenia and hemoglobin level of < 5mg/dl. We explored 1907 records, finding 907 malaria cases (50.9%) of which 188 severe non drepanocytaire cases (9.8%). Of these cases, 184 (97.8%) had hemoglobin level ≤ 5g/dl. There were 24 deaths (12.7%) of which about half occurred due to shortage of blood supply. The mortality rate was not different between females and males. However, younger children (< 3 years) died at a higher rate than their older counterparts. There is a need to increase the survival rate of younger children, notably by improving access to medical care and to blood supply.

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EVALUATION OF THE EXTERNAL QUALITY ASSURANCE PROGRAM IN 23 DISTRICTS IN UGANDA

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The World Health organization recommends parasitological diagnosis with microscopy or rapid diagnostic tests (RDT) for malaria and emphasizes with-holding antimalarials for patients with negative tests implying need for high accuracy for malaria laboratory diagnosis. Good-quality malaria microscopy requires technically competent personnel, high-quality supplies, microscopes, adequate workplace environment and an effective external quality assurance (EQA) system. The Stop malaria Project in collaboration with the Ministry of Health implemented and evaluated an EQA system in 22 districts in Uganda. The WHO based EQA System was introduced in health facilities following a refresher training in malaria diagnosis by microscopy and RDT's in 22 districts. Each health facility randomly collected two blood slides per day (a positive and a negative); of these 10 positive and 10 negative slides were randomly selected per month and sent for external reading by two expert malaria microscopists. Results for facilities were compared with those of expert district level microscopists. Discordant slides were tie broken by expert laboratory technologists at the Infectious Diseases Institute. Blinding of readers was done at all levels. The Kappa statistic was used to measure accuracy. Facilities received additional laboratory supplies to cater for stock outs. Facilities without microscopes received new ones and faulty microscopes were repaired. A total of 1876 blood slides were read; of these 141 were discordant at the district level. Forty five percent (45%) of the laboratories had excellent accuracy (excellent agreement, Kappa >0.80) 41% had good accuracy (good agreement Kappa of o.6-0.80, 5% had fair accuracy (Kappa = 0.57) while 9% had very poor accuracy (Kappa <4.0).Accuracy of reading blood slides varied across districts and this necessitates maintenance of EQA and external quality control systems in all districts to support the recent WHO malaria case management policy which emphasis parasite based diagnosis and discourage presumptive treatment with antimalarias.

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COMPARISON OF THE BINAXNOW® MALARIA RAPID ANTIGEN ASSAY TO REAL-TIME PCR AND GIEMSA-STAINED BLOOD SMEAR FOR THE DIAGNOSIS OF *PLASMODIUM* SPP. INFECTION

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Malaria continues to be a global health burden with approximately 250 billion cases and one million deaths annually. This is partially due to the fact that individuals living in resource-limited countries are the most vulnerable to infection and effective laboratory diagnostic methods and treatment in these areas is limited. Although laboratory tests such as real-time PCR and microscopic examination of whole blood smears are sensitive and specific, these methods are either expensive or require highly trained personnel. The goal of this study was to evaluate a recently FDA-approved immunochromatographic assay (BinaxNOW® Malaria, Inverness Medical, Princeton, NJ) and compare the performance of this rapid antigen test to real-time PCR and routine blood smear examination. Whole blood samples (n=157) submitted to our reference laboratory for malaria real-time PCR and/or routine blood smear were also analyzed by the BinaxNOW rapid antigen assay. Among the 157 samples tested by BinaxNOW, 101 (64%) were tested by PCR, 109 (69%) were analyzed

by blood smear, and 53 (33.7%) were tested by all three assays.When compared to real-time PCR, the BinaxNOW assay demonstrated a percent agreement, sensitivity and specificity of 92.1% (93/101), 77.4% (24/31) and 98.5% (69/70), respectively. When compared to routine blood smear, the BinaxNOW showed a percent agreement, sensitivity and specificity of 91.7% (100/109), 83.3% (25/30) and 94.9% (75/79), respectively. Interestingly, among the 4 samples that were BinaxNOW positive, smear negative, 3 (75%) were also positive by real-time PCR. These results indicate that the BinaxNOW Malaria assay could be used in emergency or point-of-care settings to rapidly rule-in malaria based on the high specificity (≥94%) and positive predictive value (≥96%) of this test. However, a negative result by the BinaxNOW assay does not rule-out malaria, and should be followed up with a blood smear or real-time PCR. The BinaxNOW assay requires less technical expertise than blood smear examination, is less expensive compared to PCR, and provides a more rapid turn-around time (15 min. *vs*. 1.5 h for blood smear *vs*. 2 h for PCR).

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A HIERARCHICAL SYSTEM TO ENSURE QUALITY OF MALARIA MICROSCOPY IN MALI

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In 2010, Mali's National Institute for Public Health Research (INRSP), with support from the Improving Malaria Diagnostics project financed by the US President's Malaria Initiative, rolled out an Outreach Training and Supportive Supervision (OTSS) program consisting of quarterly visits to health facilities to identify and correct barriers to quality malaria microscopy (MM) and malaria Rapid Diagnostic Tests (mRDTs). The baseline visits to 50 laboratories found that 38% lacked standard operating procedures (SOPs) for conducting MM; 84% had no SOPs for external quality assurance (EQA) of MM; 70% did not save slides for EQA; 86% did not keep records of internal quality assurance (QA) exercises; and 78% lacked slide boxes to preserve slides for later EQA. OTSS visits included two layers of EQA for MM: in addition to re-reading slides by laboratory supervisors during the OTSS visit, there was a second blind re-reading at INRSP. Data from 2054 EQA slides was entered into a database and analyzed with SPSS. OTSS visits provided on-the-job training to 471 microscopists and 439 clinicians. The % of laboratories in visits 1 and 4 without functioning microscopes was 6% and 0% respectively, the % experiencing stock-outs of essential diagnostic supplies in the same period was 20% and 3%; the % with MM in full agreement with national guidelines was 76% and 91%; the % using mRDTs in full agreement with national guidelines was 59% and 85%. Analysis of sensitivity (Se) and specificity (Sp) of microscopists (Mi) and their supervisors (Su) was, respectively: Se-Mi=82%, Sp-Mi=76%, Se-Su=81%, Sp-Su=90%. 80% of Su's false positives (FPs) overlapped with Mi's FPs. 80% of laboratories met a quota of 30 EQA slides per year, sufficient to calculate agreement at lab level. INRSP will compare the sustainability of an EQA scheme that sends only a sub-set of slides to INRSP for confirmation vs. proficiency testing of supervisors; Sus continue testing Mi's performance; retrain Mis and Sus not performing; and use blinding procedures to ensure that Sus are not influenced by Mi's results.

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INCREASED USE OF MALARIA DIAGNOSTIC TESTS IMPROVES TARGETING OF ANTI-MALARIAL TREATMENT IN RURAL TANZANIA: IMPLICATIONS FOR A NATIONAL ROLL OUT OF MALARIA RAPID DIAGNOSTIC TESTS

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The World Health Organization recommends diagnostic confirmation of all uncomplicated malaria cases. Tanzania is implementing a phased roll-out of malaria rapid diagnostic tests (mRDTs) in all levels of health care facilities as one strategy to increase parasitological malaria diagnosis. We evaluated artemisinin combination therapy (ACT) prescribing patterns in febrile patients with and without uncomplicated malaria in two areas with high and low levels of mRDT implementation (mRDT area and non-mRDT area, respectively). We conducted repeat cross sectional health facility surveys in two areas with health and demographic surveillance systems during both high and low malaria transmission seasons in 2010. We collected clinical information and a reference blood slide on all patients presenting for an initial illness consultation. Uncomplicated malaria was defined as fever and asexual *P. falciparum* parasitemia on a reference blood slide. We included 1,247 febrile patients in the analysis. In the mRDT area, 65% (95% confidence interval (95% CI): 52-76) of febrile patients received a diagnostic test compared to 50% (95% CI: 39-61) of patients in the non-mRDT area (p<0.001). In the mRDT area, 79% (95% CI: 66-88) of patients with uncomplicated malaria received recommended treatment with ACT compared to 67% (95% CI: 54-78) of patients in the non-mRDT area (p=0.11). Overtreatment with an ACT of patients without slide confirmed uncomplicated malaria was significantly less common in the mRDT area (23%; 95% CI: 17-30) compared to the non-mRDT area (35%; 95% CI: 29-43) (p=0.01). In conclusion, routine implementation of mRDTs resulted in increased diagnostic test use and reduced overtreatment with ACTs in one area in Tanzania. The national rollout of mRDTs will have to be monitored to assess whether these changes in case management practices will be replicated in other areas.

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OVER AND UNDER-USE OF ARTEMISININ BASED COMBINATION THERAPY AT PUBLIC HEALTH FACILITIES IN THREE REGIONS OF TANZANIA

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Artemisinin based combination therapy (ACT) is the first line drug in most malaria-endemic countries, but there are concerns that quality of care remains poor. Patients needing ACT often do not receive it, but there is also considerable over-treatment due to the lack of accurate diagnosis and inappropriate management. Tanzania is scaling up the use of rapid diagnostic tests (RDTs) to improve treatment of febrile illness. We conducted health facility surveys before RDT scale up to assess current treatment practices. We enrolled 1779 patients at 145 randomly selected health facilities in Mwanza, Mbeya, and Mtwara Regions between May and October 2010. Patients with fever in the previous 48 hours were enrolled on arrival and interviewed following their consultation. Data were collected on patient characteristics, previous treatment for fever, and care. Fingerprick blood samples were taken by study staff to test for

malaria parasitemia. Overall, 66.6% of patients attended a facility with any ACT in stock and 28.6% a facility with all 4 weight-specific doses of ACT available. 60.6% of patients had been seen by a health worker trained in ACTs. Only 6.3% of patients sought treatment at a facility that had RDTs in stock, and 5.7% saw a health worker who had been trained in RDTs. Overall, 9.8% of patients received a diagnostic test at the health facility; 82% of those tested received a blood smear and 18% an RDT. Of those tested, 54.8% were reported to have a positive test. ACTs were obtained by 58.5% of patients with a positive test, 11.4% of patients with a negative test, and 36.0% of patients who did not receive a diagnostic test during their consultation. Study RDTs conducted in all enrolled patients found that 24.5% of all patients had a positive RDT. ACTs had been obtained by 44.3% of patients with a positive RDT and 33.7% of patients with a negative RDT. Over-diagnosis of malaria remains common, with ACTs frequently prescribed to parasite-negative patients; it is anticipated that national scale up of RDTs should address this issue to some degree. However, under-treatment also remains a key problem, reflecting both ACT stockouts and inappropriate health worker practices.

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IMPROVED SEMI-NESTED MULTIPLEX PCR (SNM-PCR) FOR THE IDENTIFICATION OF THE FIVE HUMAN *PLASMODIUM* SPECIES

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Since the recognition of *Plasmodium knowlesi* as the fifth human *Plasmodium* species in Southeast Asia, cross-hybridization of the current most used PCR to identify *P. knowlesi* have been reported. Continuous attempts are made to improve the diagnosis through the development of new molecular techniques. In this report, a primer set for the identification of *P. knowlesi* in a semi-nested multiplex PCR was designed and validated against earlier published PCR based protocols. We aimed to take great advantages of the semi-multiplex PCR in detecting four popular human malarial species as published by Rubio et al, to develop a new nested PCR to identify *P. knowlesi*. Sequencing of both primary and nested PCR products were done with the view to confirming the primers amplified exactly genes of *P. knowlesi*. Human malaria species DNA, some of non-plasmodium DNA, field samples collected during malarial surveys in Ninh Thuan and Quang Nam provinces, Viet Nam were tested by three different protocols to make comparison of specificity and sensitivity. The new PCR protocol specifically amplified *P. knowlesi*, and PCR products had a band size of 500bp. No other monkey malaria strain or related was amplified by this PCR. Results were confirmed by sequencing confirming the PCR's specificity for *P. knowlesi*. that the new PCR was able to identify all *P. knowlesi* infections in our test. This protocol had a similar sensitivity and specificity with Imwong's protocol both performed better than the currently most used PCR to identify *P. knowlesi* as published by Singh et al 2004. This new protocol had as advantage to less labor intensive when analyzing for all 5 *Plasmodium* species infecting human was more economically with the Taq polymerase. Developments of new protocol starting from Rubio et al 2002 showed great number of advantages in identifying *P. knowlesi* in humans. It not only overcome cross- hybridization of the primers used with *P. vivax* - a well known problem of Singh's protocol, showed similar results with Imwong's protocol. Moreover, the success of new protocol contributes to reduce workload, time consuming and the cost of PCR technique in screening *Plasmodium* parasite in humans by sharing the same primary products for both SnM-PCR and nested PCR in detecting *P. knowlesi*.

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QUALITY ASSURANCE OF MALARIA DIAGNOSIS IN HAITI: PRELIMINARY RESULTS OF A PILOT IMPLEMENTATION IN THREE REGIONS, AUGUST 2010 - FEBRUARY 2011

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In Haiti, malaria diagnosis has relied on microscopy for many years. Following the January 2010 earthquake, rapid diagnostic tests (RDTs) were introduced in the emergency setting. The National Malaria Control Program and National Public Health Laboratory (LNSP) approved 3 *Plasmodium falciparum*-specific RDTs for use in Haiti and implemented a pilot malaria diagnostics quality assurance (QA) program in 3 regions to monitor field performance of RDTs and support microscopy. From August 2010 - February 2011, HRP2-based RDTs (CareStart™ Malaria HRP2) were introduced in 15 health facilities. Quality assurance teams from the LNSP conducted training on RDTs and introduced QA procedures. QA teams returned monthly to conduct on-site QA of microscopy and RDTs. For each patient tested by microscopy or RDT, QA personnel performed a separate blood smear for comparison. Data were reviewed and discrepancies between health facility and QA results prompted a second on-site reading of the QA blood smear. All QA smears were reread at the LNSP. 494 patients were tested for malaria during QA team visits from September 2010 - February 2011. Most sites received 3 QA visits, but activities were disrupted in October and November 2010 due to cholera response and security concerns around the Presidential election. Among the 290 blood smears performed, 55 were positive (18.9%) and 235 were negative. There were 3 false positive results and 4 false negative results, sensitivity= 92.9% and specificity= 98.7%. 465 RDTs were also performed and 105 (22.6%) were positive and 360 were negative. There were one false positive RDT result and 2 false negative results, sensitivity= 98.1% and specificity= 99.7%. QA team readings showed a high degree of internal consistency with just 1 discrepant reading. The pilot QA program demonstrated excellent performance of both RDTs and field microscopy and will be expanded to a total of 5 regions in 2011. The program was well-received by participating sites, but has high logistics and human resource demands which may be difficult to support during periods of crisis.

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NOVEL PCR-BASED ASSAYS FOR THE DETECTION OF *PLASMODIUM KNOWLESI*

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Recent studies in Southeast Asia have demonstrated zoonotic transmission of *Plasmodium knowlesi* to humans. This simian malaria parasite naturally infects long-tailed (*Macaca facsicularis*) and pig-tailed (*M. nemestrina*) macaque monkeys in much of Southeast Asia. It has a 24-hour asexual blood stage growth cycle that can lead to rapid increases in parasitemia, severe disease and, in a few human infections, has been fatal. As such, *P. knowlesi* infection requires immediate diagnosis and treatment. Microscopically, *P. knowlesi* exhibits stage-dependent morphological similarities to *P. malariae* and *P. falciparum*. These similarities have contributed to misdiagnosis of *P. knowlesi* as *P. malariae or P. falciparum*. PCR based molecular diagnostic tests were required to accurately detect

P. knowlesi in humans. The current PCR-based assay is based on the 18s ribosomal DNA sequences and can cross-react with *P. vivax* and other simian *Plasmodium* species (unpublished data). As such, we initiated the development of new PCR-based tests. In order to develop species-specific diagnostic tools for malaria, we have developed a bioinformatics approach to mine the available genome data and identify suitable DNA sequences that are highly specific to a given species of malaria parasite. Using this approach, we have identified highly specific, multicopy *P. knowlesi* sequences. We designed novel *P. knowlesi* primers for a single tube non nested PCR method. We show that this method has 100% specificity for the detection of *P. knowlesi* using three different strains of *P. knowlesi* (Nuri, H, and Hackeri) and one *P. knowlesi* infected patient specimen. In addition, no cross reactivity was observed with each of the four human malaria parasite species including 20 different strains of *P. vivax* and 5 simian malaria parasite species that were tested. This novel PCR assay is a suitable alternative for the accurate diagnosis of *P. knowlesi*. Additional laboratory and field-based testing of this assay will be necessary to validate its utility for clinical diagnosis of *P. knowlesi*.

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DEVELOPMENT OF A NOVEL CHEMICAL SERIES WITH ACTIVITY AGAINST BOTH BLOOD- AND LIVER-STAGES OF *PLASMODIUM FALCIPARUM*

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Recent progress toward the development of a novel compound series with promising *in vitro* efficacy against both blood- and liver-stage *Plasmodium falciparum* will be described. Following the failure of one such compound to cure malaria-infected mice, focus has been to enhance the pharmaceutical properties of the compound series. Newer analogs, incorporating structural changes expected to enhance these properties, have been prepared. The *in vitro* efficacies and pharmaceutical properties of these will be described. A thorough investigation of the pharmaceutical properties of representative members of the series has been undertaken, focusing on predicting their likely metabolic stability. These results, including those concerning the kinetics of *in vitro* microsomal degradation, and the subsequent identification of predicted metabolites, will be discussed.

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DEVELOPMENT OF A *PLASMODIUM FALCIPARUM* TRANSGENIC LINE FOR SCREENING DRUGS TARGETING GAMETOCYTE BY USING STRONG GAMETOCYTE SPECIFIC PROMOTER

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Malaria remains to be a devastating infectious disease, causing an estimated 500 million cases and 2 million deaths per year. Whereas the asexual stage is responsible for clinical disease, gametocytes are responsible for transmission from human host to vector. There is renewed acknowledgement that targeting gametocytes is essential for malaria control and elimination efforts. It is known that *Plasmodium falciparim* gametocytes (especially the mature stage) are relatively insensitive to many anti-malaria drugs; thus new drugs that can safely eliminate gametocytes or block transmission are needed urgently. We established a parasite cell line with the expression of the green fluorescent protein (GFP) under the gametocyte-specific promoter alpha-tubulin II. Our analysis showed GFP signals appeared in all gametocyte with relatively lower intensity in female gametocytes. The GFP signals increased from early gametocyte to mature stage (from stage I to V, even in stressed schizonts). The GFP expression was high enough to be detected by flow cytometry as early as stage 2. Based on this result, we are investigating the effect of several antimalarial drugs on gametocytes.

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N-ACETYLCYSTEINE CO-ADMINISTERED WITH PRIMAQUINE IN MALARIA TREATMENT IN ASSESSING PRIMAQUINE EFFICACY AND HEMOLYTIC TOXICITY

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Primaquine (PQ) is the only medication approved for a radical cure of malaria caused by *Plasmodium vivax or P. ovale* but toxic, hemolytic side effects in G6PD deficient individuals limits its usage. Because the toxicity of PQ may be due to oxidative stress induced by its reactive metabolites, we investigated whether N-Acetylcysteine (NAC), which has potent anti-oxidant activity, could attenuate the PQ-mediated oxidative stress without compromising efficacy. We co-administered NAC and PQ in the following animal models: the causal prophylaxis *P. berghei* efficacy model, utilizing *In vivo* Imaging Spectrum (IVIS), and the C3H G6PD deficient rodent screening model. Both are used to screen potential antimalarial drug candidates. In the efficacy model, 10 mice were given PQ 10mg/kg orally once daily and NAC 200mg/kg twice daily by intraperitoneal injection for 3 days. Luciferase expressing *P. berghei* sporozoites were inoculated on day 2. Images were obtained daily for 3 days and blood samples obtained for parasitemia every 2 days for a total of 2 weeks. In the G6PD deficient rodent model doses used were PQ 8.75 mg/kg given daily with or without NAC 400mg/kg administered twice daily both orally for 5 days. Hematologic parameters were tested at baseline and day 6 to detect hemolysis. In the efficacy model, no inhibition of activity was seen by, but 3/5 animals in the control and 2/5 in the experimental group died during the parasitemia follow up. These limited data did not show an effect of NAC on PQ efficacy. In the G6PD deficient model (n=8), there was no difference in hemolytic endpoints between groups (Mean change Mature RBC (M/ml), PQ only = -1.6, PQ+NAC = -1.8). Preliminary results suggest that low dose NAC produced no significant difference between the two groups in either model. Further testing of both drugs, at different doses in both models is on-going; results will be available for presentation.

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EFFICACY OF DIFFERENT NITRIC OXIDE-BASED STRATEGIES TO PREVENT EXPERIMENTAL CEREBRAL MALARIA

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The high case-fatality rate and morbidity of cerebral malaria despite parenteral antimalarial therapy incites investigators to develop preventive and adjunctive therapies for the disease. Preclinical studies using experimental models of cerebral malaria are useful to understand its pathophysiological processes and can be a first step to test therapies for the condition. Low nitric oxide (NO) bioavailability plays a role in the pathogenesis of experimental cerebral malaria (ECM) caused by *Plasmodium berghei* ANKA. The disease is prevented by the treatment with a high concentration of the NO donor dipropylenetriamine NONOate (DPTA-NO). However, it is not known how NO acts to prevent the development of ECM and if more physiologically and clinically relevant treatments aiming to improve its endogenous synthesis or effects through the generation of cyclic guanosine monophosphate (cGMP) would also show efficacy in the model. We studied the efficacy and safety of different strategies to improve NO bioavailability in preventing the development of ECM. Treatments with L-arginine, an arginase inhibitor (N-hydroxy-nor-Arginine) and tetrahydro-L-biopterin when given alone or in different combinations aiming to optimize the endogenous synthesis of NO through

the L-arginine-nitric oxide synthase-NO pathway were not efficient to prevent ECM. Prevention of ECM was neither achieved with sodium nitrite treatment, indicating that the phenomenon of nitrite reduction to NO may not play a significant role in the pathogenesis of the disease. Finally, treatment with low doses of DPTA-NO or the inhibition of the enzyme phosphodiesterase-5 with sildenafil did not prevent ECM, but a significant decrease in mortality was observed when both strategies were combined. The combined therapy did not cause anemia or hypotension, which are major side effects generated by the treatment with high doses of DPTA-NO. We conclude that therapies targeting the NO-sGC-cGMP pathway for ECM are feasible, but have to be optimized to decrease potential side effects caused by the administration of NO.

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AN *IN VIVO* GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PD)-DEFICIENT MOUSE MODEL TO PREDICT HEMOLYTIC TOXICITY OF CANDIDATE 8-AMINOQUINOLINE (8-AQ) ANTIMALARIAL DRUGS

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Determination of hemolytic potential of 8AQ candidate compounds in animal models prior to commitment of resources for human clinical trials would be invaluable for the development of new 8AQ drugs with a higher therapeutic index. We developed a G6PD-deficient (G6PDD) mouse model that demonstrates a phenotype similar to human African type A^- population (10-15% of normal G6PD activity). We qualified this mouse model by testing three known hemolytic 8-AQs, *i.e.*, primaquine (PQ), pamaquine (PaQ) and tafenoquine (TQ), and two known non-hemolytic drugs, chloroquine (CQ) and mefloquine MQ). G6PDD mice given the hemolytic drugs consistently displayed all hemolytic parameters. The decreases in mature RBC counts (16 - 64%) in G6PDD mice in response to PQ (22 - 88 mg/kg) were dose- dependent. PaQ (70 mg/kg) and TQ (40 mg/kg) also decreased mature RBC counts (67% and 8%, respectively). Results indicated that the reticulocyte production and Heinz body formation were triggered in G6PDD mice by PQ, PaQ and TQ. Neither clinical signs of hemolysis nor obvious changes in hemolytic parameters were observed under the same experimental conditions by using CQ and MQ. We evaluated this mouse model with two regimens of dosing, one of 6 days duration and one of 10 days duration, with similar results. We also developed a hemolytic index (HI) to compare the hemolytic potential between 8-AQs, using PQ as the reference standard. In summary, our results demonstrate that the G6PDD mouse model appears to be useful in predicting the hemolytic toxicity of new 8-AQs and potentially other hemolytic drugs. This *in vivo* model has the attributes of: 1) employing a genetically altered, but otherwise normal G6PDD condition; 2) having a phenotype similar to the African type A^- G6PDD patients; 3) exhibiting a hemolytic toxicity response to 8-AQs; 4) showing no hemolytic response to two non-hemolytic antimalarials, and 5) displaying reproducible and statistically valid assay results. We now intend to utilize this new tool to identify safer 8-AQs.

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INHIBITION OF *PLASMODIUM FALCIPARUM* CALCIUM DEPENDENT PROTEIN KINASE 4 PREVENTS MOSQUITO INFECTION AND MALARIA TRANSMISSION

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Current antimalarial drugs allow continued transmission of malaria from infected individuals to mosquitoes after successful therapy. Effective control and eradication of malaria will require new tools to prevent transmission of these parasites. *Plasmodium* calcium dependent protein kinase 4 (CDPK4), shown to be essential for exflagellation of microgametes, sexual reproduction and infection of the mosquito host, is a promising target because orthologs are absent in mammalian genomes. CDPK4 has a serine at the gatekeeper position of the ATP binding site which renders CDPK4 sensitive to bumped kinase inhibitors (BKIs). We describe BKIs that inhibit *Plasmodium falciparum* calcium dependent protein kinase 4 (PfCDPK4) and block the infection of mosquitoes with malaria-parasites from mammalian-hosts. A BKI that has activity against PfCDPK4 prevents the exflagellation of *P. berghei* mouse malaria-parasites that express PfCDPK4. Administration of the BKI compound to mice stops the transmission of malaria to mosquitoes. Finally, addition of the BKI compound to blood with *P. falciparum* gametocytes stops exflagellation of microgametocytes and blocks the sexual-stage in mosquitoes. These compounds have a low likelihood to select resistance as the selective pressure for selection of resistance is only manifest in the mosquito gut, where natural infection involves only 2 to 10 gametocytes. Our studies thus far indicate that this strategy leads to non-toxic, selective inhibitors that block malaria transmission to mosquitoes, have favorable oral pharmacokinetic (PK) properties, and are thus excellent leads for further drug development. This series of BKI compounds will be further optimized, through structure based drug development and PK measurements, to achieve transmission blocking exposure during the life of the gametocyte. These compounds could be valuable in malaria control and eradication programs.

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POTASSIUM CHANNELS AS DRUG TARGETS IN *PLASMODIUM* PARASITES

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Potassium channels are integral membrane proteins devoted to regulation of membrane potential and cell volume. Malaria parasites encode two K^+ channel homologues (Kch1 and Kch2), which are well-conserved among members of the *Plasmodium* genus. In the rodent malaria parasite *P. berghei*, the two K^+ channel homologues PbKch1 and PbKch2 were studied using targeted gene knock-out. First, the transgenic parasites were characterized in a mouse model in terms of growth-kinetics and transmission potential. Second, using a tracer-uptake technique and $^{86}Rb^+$ as a K^+ congener, the K^+ transporting properties of the transgenic parasites were assessed. Third, the impact on parasite membrane potential of the two K^+ channels was investigated using a potential-dependent fluorophore DiBAC4 bis-oxinol. Results: *i)* Knock-out of either K^+ channel did not grossly affect the phenotypes in terms of asexual replication and

pathogenicity in mice. Though, *P. berghei* parasites deficient in PbKch1 (PbKch1-null parasites), but not PbKch2-null parasites, were unable to form oocysts in female *Anopheles stephensi* mosquitoes. *ii)* PbKch1-null parasites, but not PbKch2-null parasites, had a low $^{86}Rb^+$ uptake, when compared to wild-type (WT) parasites. The Kch1-mediated $^{86}Rb^+$ uptake was inhibited by K^+ channel blockers; the residual, non-Kch1-mediated, $^{86}Rb^+$ uptake was not sensitive to further inhibition by K^+ channel blockers. *iii)* Kch1, but not Kch2, apparently influenced the membrane potential of the parasites. In conclusion, our studies suggest unequivocally that the *Plasmodium* K^+ channel 1 is a functioning K^+ channel, which contributes to the K^+ permeability of the parasites plasma membrane. The channel is, for yet unknown reasons, necessary for sexual replication of *P. bergehi parasites* in the mosquito midgut. These studies provide a rationale for pharmacological inhibition of the Kch1 orthologue in human parasites as a novel strategy to disrupt malaria transmission.

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EFFECTS OF DHA-PIPERAQUINE ON THE QTC INTERVAL IN NORTHERN CAMBODIA

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To date there is not good evidence of clinically significant QT prolongation with piperaquine at therapeutic doses. Effects of DHA-piperaquine treatment on the EKG were assessed as part of an active observational cohort study of malaria epidemiology in healthy volunteers taking either 2 or 3 days of DHA-piperaquine in Northern Cambodia in 2010-2011. A total of 80 subjects were randomized to open label DHA-piperaquine using the same cumulative treatment dose currently recommended by WHO (360mg/2880mg) with either 2 or 3 days of dosing. 12 lead EKGs were obtained at screening, pre-dose, daily for 3 days, and then weekly for 4 weeks if QT prolongations >10ms were seen during the dosing period. A high proportion of subjects (8%) were excluded at screening from the cohort study based on QTc Bazett (QTcB) prolongations greater than 500ms. The mean increase in QTc interval following dosing was 5-6% over baseline by QTcB, and 6-8% by QTc Fridericia (QTcF). Only 2 of 80 volunteers had a prolonged QTcF greater than 20% over baseline and, in both cases, this was observed on a single day during the 6 week follow-up period. By QTcF, there were 7 AEs for QTc prolongation (17.5%) in the 3 day group by CTC AE v4.0 (4 grade 1 and 3 grade 2) vs. 8 in the 2 day group (20%) (7 grade 1 and 1 grade 3). There were not significant differences in QT prolongation between treatment groups. In many cases, prolongation was present at baseline or clearly influenced by the confounding effects of fever, malaria and increased heart rate. Piperaquine as part of a DHA-piperaquine combination caused modest QTc prolongation at treatment doses over 2 or 3 days, and the effect was similar to what has been reported in other studies. Further evaluation of the potential for QTc prolongation, particularly with repeated dosing is needed, as is the epidemiology of acquired and congenital long-QT syndromes in this population, given the high rate observed in this cohort. PK-PD analysis will be presented once PK analysis has been completed.

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TRIAGE OF HIGH THROUGHPUT SCREENING RESULTS AGAINST BLOOD STAGE *PLASMODIUM FALCIPARUM*

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A 500 member natural product library was assayed for activity against blood stage *Plasmodium falciparum* using a SYBR Green I-based fluorescence (MSF) assay, as reported previously. The screen was performed at a single concentration (10,000 ng/mL); from these initial results, IC_{50}s were determined against three plasmodium strains (D6, W2 and C235) for 40 compounds of interest. Nine compounds that met specific potency criteria against three *Plasmodium* strains were advanced to further studies in our malaria drug discovery testing strategy. Our testing strategy for discovery of new anti-malarial compounds, the results of our *in vitro* campaign and progression of several key compounds into an *in vivo* blood stage mouse malaria model will be presented.

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THE NONSELECTIVE CYTOCHROME P450 INHIBITOR, 1-AMINOBENZOTRIAZOLE, DOES NOT PREVENT PRIMAQUINE-INDUCED HEMOLYSIS IN A HUMANIZED NOD-SCID MOUSE MODEL OF G6PD DEFICIENCY

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The 8-aminoquinoline drugs primaquine (PQ) and tafenoquine are critical to malaria elimination efforts given their anti-relapse and gametocidal activity. Realizing the full potential of these drugs is impeded by drug-induced hemolytic toxicity in glucose-6-phosphate dehydrogenase deficient (G6PDd) individuals. We are exploring methods to improve the safety of this drug class.We previously showed that 1-aminobenzotriazole (ABT), a nonselective irreversible inhibitor of multiple cytochrome P450s (CYP450s), blocked the causal prophylaxis of PQ in a *Plasmodium berghei* sporozoite challenge mouse model. ABT inhibits CYP450 isoforms to varying degrees by *N*-alkylation of the heme moiety. We subsequentlyinvestigated the effects of ABT in PQ-induced hemolysis in a humanized severe combined immunodeficiency (SCID) mouse model of G6PDd. In this model, PQ and other hemolytic drugs cause a dose-dependent hemolysis in non-obese diabetic (NOD)-SCID mice engrafted with human G6PDd red blood cells (RBCs). Mice engrafted with human A minus-type G6PD RBCs were orally dosed with 150 mg/kg ABT two hours prior to oral dosing with 6.25 or 12.5 mg/kg PQ for 3 days. Comparator groups were treated with the same doses of PQ and ABT alone. After 7 days, PQ alone caused a dose-dependent loss of human RBCs and increase in mouse reticulocytes. Hemolysis did not occur in A minus G6PDd humanized mice treated with ABT alone and pre-treatment with ABT did not block PQ-induced hemolysis. These preliminary results suggest that CYP-mediated metabolites of PQ may not be responsible for inducing hemolysis in G6PDd. We are confirming the effect of ABT on PQ-induced hemolysis in NOD-SCID mice and a mutant G6PDd mouse model. Studies are also underway to confirm no effect of ABT on non-CYP pathways. The results attained thus far in SCID mice, along with our previous results indicating that ABT inhibits PQ's malaria prophylaxis activity in mice, suggest that we may be able to develop a strategy for improving the therapeutic index of 8-aminoquinolines.

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POPULATION PHARMACOKINETICS OF PYRONARIDINE FOLLOWING ORAL PYRONARIDINE/ARTESUNATE TREATMENT IN HEALTHY AND MALARIA INFECTED SUBJECTS

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Artemisinin-based combination therapies (ACTs) are currently recommended by the World Health Organization as a first-line treatment for uncomplicated *P. falciparum* malaria. Pyronaridine is a promising ACT partner drug as shown by its efficacy against antimalarial-resistant strains. This has led to the development of a new antimalarial drug, pyronaridine/artesunate (PA) fixed-dose combination. The population pharmacokinetics of pyronaridine are reported using data from healthy (166) and malaria infected (642) subjects participating in nine Phase I-III clinical trials. Pyronaridine blood concentrations were measured using validated HPLC and LC-MS/MS methods. Non-linear mixed effect modeling approach was performed to investigate the influence of nine covariates (age, weight, body mass index, malaria infection, creatinine clearance, alanine aminotransferase, aspartate aminotransferase, gender, and ritonavir administration) on the pharmacokinetics of pyronaridine. A two-compartment model with first-order absorption and elimination adequately described pyronaridine pharmacokinetics. After the inclusion of statistically significant covariates, the population parameter estimates of apparent clearance (CL/F), central volume of distribution (V2/F), peripheral volume of distribution (V3/F), apparent inter- compartmental clearance (Q/F) and absorption rate constant (Ka) were 434 L/day, 907 L, 4,430 L, 1,120 L/day and 16.7 day^{-1}, respectively. The corresponding inter-individual variability estimates for CL/F, V2/F, V3/F, Q/F and Ka were 53.6%, 103%, 29%, 28.8% and 67.5%, respectively. The elimination half-lives of pyronaridine in healthy adult subjects, adult and pediatric malaria subjects were estimated to be 11.3, 13.2, and 9.6 days, respectively. Malaria infection was a significant predictor for V2/F and CL/F and weight was a significant predictor for V3/F and CL/F. Model evaluation results showed that the final model is robust, predictive and stable as confirmed by non-parametric bootstrap, visual predictive check and condition number.

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ESTIMATION OF THE COMPARATIVE HEMOLYTIC POTENTIAL OF PRIMAQUINE AND ANALOGS IN A HUMANIZED NOD-SCID MOUSE MODEL OF G6PD DEFICIENCY

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8-aminoquinolines (8-AQs), of which primaquine (PQ) is the prototype, are critical to malaria elimination efforts, given their anti-relapse and gametocidal activity. Clinical use of 8-AQs is impeded by hemolysis in glucose-6-phosphate dehydrogenase deficient (G6PDd) subjects. A critical need is animal models in which human G6PDd sensitivity to PQ can be reproduced. We have previously reported a model in which 8-AQs cause hemolysis in NOD-SCID mice engrafted with human G6PDd RBCs. In this model, direct comparison of hemolytic potency of analogs with PQ is feasible, but fails to take account of the comparative efficacy. We therefore developed an approach which first estimates the effective causal prophylactic (CP) dose in an ICR mouse *Plasmodium berghei* (ANKA strain) sporozoite challenge model. In the CP model, drugs are dosed for 3 days (-1, 0, and 1) with sporozoite inoculation given on d 0; untreated mice succumb to infection after several days. 8-AQs are highly effective in this model with an effective dose (ED)100 for PQ at 25 mg/kg/d given orally for 3 d. In the NOD-SCID hemolytic model, this CP ED100 dose of PQ yields loss of about 75% of human G6PDd (A- genotype) RBCs by d 7. To generate a normalized PQ hemolytic dose-response curve to compare efficacy and toxicity, we did a hemolytic dose response curve at multiples of the CP ED 100. Thus, a new 8AQ analog's ED100 can be established in CP model, then the hemolytic potential of PQ and the analog can be directly compared in the huRBC SCID model at equi-effective CP doses. NPC1161B, an 8-AQ development candidate which is a pure (-) (*R*) enantiomer, has a 3-d ED100 in the CP model of 0.5 mg/kg, while at this dose elicits no hemolysis in the huRBC SCID model. In contrast, NPC1161A, the opposite (+) (*S*) enantiomer, has a 3-d ED100 of 8 mg/kg, and at this dose it is indistinguishable from PQ with regard to hemolysis. This result indicates that hemolytic potential can be reduced without compromising efficacy in 8-AQs.

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THE PHARMACOKINETICS AND PHARMACODYNAMICS OF (+) AND (-) PRIMAQUINE ENANTIOMERS IN HEALTHY RHESUS MONKEY MODEL

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Primaquine (PQ), the standard drug for radical cure of relapsing malaria for more than 60 years is administered as a racemate of (+) and (-) enantiomers. Differential toxicities of the enantiomers, particularly in hemolytic potential and methemoglobin (mtHb) formation could represent a pharmacodynamic advantage. While Schmidt reported in 1977 that (-) PQ caused increased liver toxicity twice that of (+) PQ, hematologic effects are less well understood. We administered oral primaquine enantiomers to healthy Rhesus macaques in dose-rising fashion at 1.3 (human-equivalent treatment dosage), 3.0 and 4.5 mg/kg/day (n = 3 per enantiomer, 1 control). Drug was administered for 7 days with a 2-week washout period between doses, and cross-over of enantiomers at the high dose. Pharmacokinetic samples were collected on day 7 of dosing at 3.0 and 4.5 mg/kg, and effects on blood, liver and kidneys were assessed. There was little appreciable hemolytic activity at any dose, and no effect on renal function. (+) PQ showed more consistent rises in mtHb, but only 1/6 animals had substantial mtHb formation (>10%), and this was observed in the same animal with both enantiomers. (-) PQ caused a reversible hepatotoxicity > 10x the upper limit of normal in 2 of 3 animals at 4.5 mg/kg. Effects of the enantiomers on both mtHb and liver function were plasma concentration-independent for the parent compound or the carboxyprimaquine metabolite which formed at 3-5x higher concentration with (-) compared to (+). There was no enantiomeric interconversion *in vivo*. In healthy Rhesus, primaquine enantiomers show divergent toxicity patterns, with hepatotoxicity associated with the (-) form, while the (+) form seems to exert more red cell oxidative stress, as reflected in mtHb generation. The relevance of the liver injury and mtHb formation for PQ clinical use are not clear, but the findings suggest that the two enantiomers have different patterns of metabolism and disposition.

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STANDARDIZED, HIGH-THROUGHPUT ANALYSIS OF *IN VITRO* ANTIMALARIAL SUSCEPTIBILITY DATA WITHIN THE WORLD WIDE ANTIMALARIAL RESISTANCE NETWORK (WWARN)

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The mission of the Worldwide Antimalarial Resistance Network (WWARN) is to provide the comprehensive, timely, quality-assured intelligence needed to track the emergence and spread of antimalarial drug resistance. *In vitro* testing remains a central pillar of antimalarial efficacy surveillance since drug susceptibility in parasites isolated directly from patients (termed ex vivo testing) is largely independent of clinical factors and hence provides current information that complements clinical assessment of drug efficacy. Moreover, isolates can be assessed to determine responses to each component in a combination therapy or to alternative drugs not in use in that location. WWARN's *In vitro* Module aims to enhance the quality, quantity and geographic extent of these ex vivo data available to the malaria community via a global data repository. In order to accommodate variations in analytical approach used by different centres, WWARN deals exclusively with primary (raw) data, allowing characteristics of methodology to be understood and analyses to be undertaken via a standardised approach. For ex vivo studies, the primary data are the output from assessment of drug effects on an individual isolate for a single drug. Here we describe the development, validation and application of data analysis tools that allow calculation of standard susceptibility parameters via non-linear regression. Data that meet prospective quality criteria can be displayed on the online WWARN Explorer map and entered into pooled analyses incorporating clinical, molecular and pharmacokinetic data.

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GENETIC DIVERSITY OF *PLASMODIUM FALCIPARUM* SARCO(ENDO)PLASMIC RETICULUM CA^{2+} ATPASE (PFSERCA) IN GREATER MEKONG SUBREGION: IMPLICATIONS FOR MALARIA CONTROL

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Recently, artemisinin combination therapies (ACT) to treat *Plasmodium falciparum* malaria have been widely used in endemic malaria endemic countries. Although the mechanism of artemisinin resistance was not clear, the *Plasmodium falciparum* Sarcoplasmic/Endoplasmic Reticulum Ca2+ ATPase (PfSERCA) has been speculated to be the target of artemisinins and thus a potential marker for resistance. Here we sequenced Pfserca gene (serca) in 213 samples from the Greater Mekong Subregion (GMS) and identified 23 SNPs, of which 13 were newly reported and 13 resulted in amino acid substitutions. Most SNPs (17/23) detected in our samples were clustered within the cytoplasmic domains of the protein. No isolates showed point mutations at codons S769N or L263E, which were reported to be associated with decreased sensitivity to artemether *in vitro*. We analyzed a worldwide sample collection of 862 *P. falciparum* isolates (19 populations from Asia, Africa, South America and Oceania) for the Pfserca gene (72 SNPs from around 3600 nucleotides per isolate). Of 110 nucleotides haplotypes observed, the ancestral haplotype (441 samples) was present in 16 populations and it was the predominant haplotype in samples from Africa, Asia and Oceania. The reference sequence haplotype (3D7) (54 samples) is the second most common haplotype, present in 9 populations from all four continents. The dN/dS ratios were below one, indicative of purifying selection. Molecular evolution studies did not detect significant departure for most of the populations. These results suggest further studies are needed to assess genetic diversity of serca in malaria endemic regions and to evaluate its usefulness in monitoring ACT sensitivity.

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MOLECULAR MARKERS OF ANTIFOLATE RESISTANCE IN *PLASMODIUM FALCIPARUM* ISOLATES FROM LUANDA, ANGOLA

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Plasmodium falciparum malaria remains a leading health problem in Africa and its control is seriously challenged by drug resistance. Although resistance to the sulfadoxine-pyrimethamine (SP) is widespread, this combination remains an important component of malaria control programs as intermittent preventive therapy (IPT) for pregnant women and children. In Angola, resistance patterns have been poorly characterized, and IPT has been employed for pregnant women since 2006. The aim of this study was to assess the prevalence of key antifolate resistance mediating polymorphisms in the *pfdhfr* and *pfdhps* genes in *P. falciparum* samples from Angola. Sixty-one *P. falciparum* samples collected in Luanda, in 2007, were genotyped by amplification and DNA sequencing of the *pfdhfr* and *pfdhps* genes. The most prevalent polymorphisms identified were *pfdhfr* 108N (100%), 51I (93%), 59R (57%) and *pfdhps* 437G (93%). Resistance-mediating polymorphisms in *pfdhps* less commonly observed in West Africa were also identified (540E in 10%, 581G in 7% of samples). This study documents a high prevalence of 4 *P. falciparum* polymorphisms that predict a significant level of antifolate resistance in Luanda. Further, a minority of samples contained additional mutations predicted to mediate high-level resistance. We concluded that the use of SP for IPT may no longer be warranted in Angola.

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WWARN MOLECULAR SURVEYOR: MAPPING THE GLOBAL DISTRIBUTION OF ANTIMALARIAL RESISTANCE MUTATIONS IN *PLASMODIUM FALCIPARUM*

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The spread of drug resistance has rendered sulfadoxine pyrimethamine (SP) ineffective for treatment of *Plasmodium falciparum* malaria in much of the malaria-endemic world. However, SP is still effective as first -line therapy in some regions, and has been shown to be efficacious as intermittent preventive therapy in pregnant women (IPTp), infants (IPTi) and children (IPTc) in many African regions with high malaria transmission intensities. SP-IPTp is being widely implemented in sub-Saharan Africa.

The World Health Organisation recently recommended SP-IPTi in African regions where SP resistance does occur but only within a specific resistance threshold. As patterns of SP use change with implementation of SP-IPT along with increased uptake of artemisinin combination therapies for curative treatment, patterns of resistance must be continuously monitored. Hundreds of studies have investigated the presence of SP resistance-conferring mutations in the dhfr and dhps genes of *P. falciparum*. In an effort to collate and display these published data freely, the WorldWide Antimalarial Resistance Network (WWARN) has created a collaboration to produce interactive web-based maps of global SP resistance mutations called Molecular Surveyor. Molecular Surveyor allows users to view the number of samples positive for a specific resistance marker along with the total number of samples tested, year of sample collection, location of survey and a web link to each original publication. Users may filter the map by drug, resistance marker, time span and sample size of surveys. Molecular Surveyor is currently driven by data from literature reviews conducted by three groups of investigators from four continents. The database has 2,460 data points from 525 sites around the globe. Future versions of Molecular Surveyor will map the distribution of other resistance markers such as pfcrt and pfmdr1.

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SURVEILLANCE OF MOLECULAR MARKERS OF SULPHADOXINE-PYRIMETHAMINE RESISTANCE IN GHANA AFTER THE CHANGE OF MALARIA TREATMENT POLICY

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In Ghana, sulphadoxine-pyrimethamine (SP) is used as intermittent preventive treatment in pregnant women (IPTp) since 2005. Before then, it was the second-line drug for the treatment of uncomplicated malaria. SP is an over the counter drug for IPTp and thus may be available for use by others. Drug pressure enhances the spread of resistant parasites and it is therefore imperative to monitor molecular markers of *Plasmodium falciparum* antimalarial drug resistance for early detection of the development of resistance. This information in addition to in-vivo and in-vitro assessment of drug resistance is crucial for treatment policy amendment and for non-immune travelers in their choice of prophylactic antimalarials. We therefore characterized mutations in *P. falciparum* dihydrofolate reductase (dhfr) and dihydropteroate synthase (dhps) for sulphadoxine- pyrimethamine (SP) resistance after the change in treatment policy in Ghana. 738 filter paper blood blots collected from 2005-2010 from children aged 6-59 months with uncomplicated malaria presenting to 9 existing sentinel sites for monitoring antimalarial drug resistance in Ghana were analyzed. PCR followed by restriction length polymorphism (RFLP) analysis was used to characterize mutations at codons 51, 59, 108, 164 of the dhfr gene and codons 436, 437 and 540 of the dhps gene. Data analysis included the determination of the prevalence of the mutations and the trend over the years. The overall trend showed an increase in the prevalence of the mutations from 2005-2010. The prevalence of the dhfr triple mutant (51, 59 and 108) was 29% for 2005-2006, 46% for 2007-2008 and 54% for 2010. No dhfr 164 mutant was observed in all the samples. The dhps double mutant (437 and 540) was 0.42%, 0.24% and 1.12% respectively for 2005-2006, 2007-2008 and 2010. For dhps double mutant (436 and 437) was 43%, 45% and 38% for 2005-2006, 2007-2008 and 2010 respectively. High prevalence of the 437 mutation (range: 59%-80%) was observed compared to low prevalence of the 540 mutant (0.24%-1,12%). The quintuple mutant (dhfr 51, 59, 108 and dhps 437, 540) was only observed in one of the 2010 samples. The study shows the effect of the continuous use of SP which enhances the selection and spread of resistant parasites in the country. Whether the use of SP alone for IPTp is justified will be further discussed.

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AMPLIFIED PFMDR1 COPY NUMBER IN *PLASMODIUM FALCIPARUM* FROM ARTEMETHER-LUMEFANTRINE TREATED PATIENTS IN EASTERN SUDAN

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Malaria remains a global health challenge being responsible for one million deaths annually world-wide. The dissemination of drug resistance from focal areas where it evolved to almost all endemic areas has fuelled increased disease burden and lead to recent changes in drug policies in sub-Saharan Africa. The pfmdr1 gene in *Plasmodium falciparum* is a candidate marker of drug resistance to antimalarial drugs with different mechanisms of action. Point mutations in this gene modulate susceptibility to 4-aminoquinolines (CQ), arylaminoalcohols (lumefantrine) and more recently artemisinins derivatives (artemether) both in clinical isolates and culture adapted laboratory clones. Increased copy number of pfmdr1 has been observed in clinical isolates from Thailand and Cambodia where mefloquine resistance is wide spread. However, there have been two reports of the occurrence of pfmdr1 amplification in Africa. The first report of a clinical isolate from Gabon collected in 1995 and the second more recent report of an isolate from Kenya collected in 2004. In this study, 74 pre-treatment and 14 post treatment isolates collected from eastern Sudan during an artemether-lumefantrine clinical trial in 2006 were investigated for amplification of the pfmdr1 gene employing a duplex hydrolysis probe qPCR assay. Fifty-seven pre-treatment isolates gave interpretable results. In these there was an increase in copy number in 10.5% of samples with a mean in this group of 1.89 copies range (1.63 to 2.33). Interestingly the isolate with the highest copy number 2.33 failed treatment on D14, but the recurrent isolate had only one copy of the gene. The second isolate with copy number of 2.07 successfully cleared parasitaemia by microscopy, however it harboured PCR detectable parasites on D14, and these also had one copy of the gene. Isolates with increased copy number were predominantly of the YFSND haplotype (N86Y, Y148F, S1034C, N1042D, D1246Y). It is clear that pfmdr1 amplification has spread to Africa in a different genetic background than that observed in South East Asia. This highlights the significance of monitoring genetic polymorphisms in pfmdr1 in the ACT era.

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INVESTIGATING THE POOR PARASITOLOGICAL PERFORMANCE OF ARTEMETHER-LUMEFANTRINE AGAINST MALARIA IN BUKOBA VILLAGE, MAYUGE DISTRICT, UGANDA

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Artemisinin-based combination therapies (ACTs) are the first-line treatments for home-based management of *falciparum* malaria in many countries. In Uganda, artemether-lumefantrine (AL) is the ACT promoted by the Ministry of Health. There are, however, reports of reduced ACT efficacy on the Cambodian-Thai border and we have encountered potential poor performance of AL in Uganda. To investigate the AL efficacy more carefully, we conducted a study in Bukoba village on Lake Victoria where prevalences of *Plasmodium falciparum*, *P. malariae* and *P.*

ovale sp. in children under six were 88%, 16% and 8% respectively. At baseline 188 children were screened using four malaria rapid diagnostic tests (RDTs), including HRPII and LDH targets. Children who were positive (n=176) by the HRPII test were treated with AL. On Day 7 children were retested with LDH tests and all positives were retreated (n=30) with AL. On Day 17 testing was repeated and RDT positives (n=20) were retreated with oral quinine. Of these, five still showed parasitaemia seven days later. Genotyping of the parasites to distinguish between recrudescences and new infections is ongoing. In addition, we are investigating mutations in the *P. falciparum* multidrug resistance 1 locus (*pfmdr1*) and chloroquine resistance transporter gene (*pfcrt*), among other genes, which could potentially be involved in the reduced drug response. Our results suggest that AL treatment is not entirely effective against malaria in this rural setting and we hope to gain an insight into the molecular basis for this.

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DRUG RESISTANCE IN *PLASMODIUM FALCIPARUM*: IDENTIFICATION OF A NOVEL ADDITIONAL PFCRT LOCUS WHICH LACKS INTRONS AND IS TRANSCRIBED *IN VIVO*

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The chloroquine resistance transporter located on the membrane of the parasite digestive vacuole is believed to influence access of chloroquine transport to its target. Data from transfection studies has proposed a role for polymorphisms in the pfcrt locus in mediating susceptibility to various antimalarial drugs, particularly the 4-aminoquinolines chloroquine and amodiaquine. Genetic studies have shown a considerable number of single nucleotide polymorphisms in this gene from various parts of the world that have been associated with susceptibility and resistance to chloroquine and more recently artemisinin combinations, particularly artemether-lumefantrine. In this study we sequenced amplified pfcrt cDNA from transcripts taken directly from clinical isolates collected during studies of artemether-lumefantrine efficacy in Eastern Sudan. Novel non-synonymous polymorphisms were observed in transcripts encoding PfCRT. Unexpectedly, from a handful of patients in one Sudanese site, splicing variants with 2 exons missing were observed. Further investigations of genomic DNA revealed complete removal of introns, and at least 2 exons, from the 3' end of a previously undescribed genomic pfcrt-like locus in these isolates. A similar phenomenon was not observed among *in vivo* cDNA sequences from 50 isolates collected from Tanzania in the same year, although novel SNP were also evident in this group of parasite isolates. A normal intron-interrupted pfcrt locus appears to also be present in the same isolates. The possible biological significance of our findings will be discussed in the context of a substantial shift in drug selection pressure away from aminoquinolines in east Africa.

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IMPACT OF SEASONAL INTERMITTENT PREVENTIVE TREATMENT IN CHILDREN: MOLECULAR MARKERS OF RESISTANCE IN THREE HEALTH DISTRICTS IN SENEGAL

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A study was conducted by the department of parasitology, IRD and the LSHTM in three health districts in central Senegal. Seasonal intermittent preventive treatment that three administrations of the combination sulfadoxine-pyrimethamine and amodiaquine is performed each month during the three month period of transmission of malaria in children under 5 years. So we evaluated mutations in pfdhfr and pfdhps genes resistance markers of *P. falciparum* to SP and pfmdr1 and pfcrt genes, markers of resistance to chloroquine and amodiaquine. This study is from January 2008 to January 2011, takes place in three sites with seasonal transmission (Mbour Bambey and Fatick). It was performed in a gradual manner with 9 areas in the first year, 27 in the second, 45 in the third year. So preliminary results showed a prevalence of mutations in codons 51, 59 and 108 gene pfdhfr turning around of 80%. Results obtained in this study showed that there was no significant difference between intervention areas and control areas. IPTc has no effect on mutations in the pfcrt, pfdhfr, pfdhps and Pfmdr genes. And as far as no mutations in codons 164 and 540 of the pfdhfr and pfdhps genes respectively. This could be a strong signal to the central question about the durability of the use of SP.

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USE OF MALARIA IMPORTED CASES IN NON-ENDEMIC COUNTRIES TO ASSESS THE RETURN OF CHLOROQUINE SUSCEPTIBILITY IN SENEGAL

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In compliance with WHO recommendations, African countries have discontinued chloroquine (CQ), due to widespread resistance, and now promote artemisinin-based combination therapy (ACT), as first-line treatment for uncomplicated malaria. Faced with an average CQ treatment failure of 25%, Senegal changed its national malaria policy in 2003 from CQ to amodiaquine (AQ) + sulfadoxine-pyrimethamine and in 2006, to AQ+artesunate. Studying travelers returning from a specific region and collectively infected by a wide variety of strains of *Plasmodium falciparum* (Pf), could be an effective tool for detecting the evolution of resistance onsite. The aim of the study is to describe the evolution of CQ resistance in Senegal after a decrease of drug pressure, through imported cases from the country. The study was conducted by the Malaria National Reference Centre in France in collaboration with the WorldWide Antimalarial Resistance Network (WWARN). We collated *in vitro* response of reference and clinical isolates for CQ with a standard 3H-hypoxanthine test and prevalence of pfcrt K76, the molecular marker for CQ susceptible Pf malaria. In total, 215 clinical isolates were tested from 1996 to 2004 and 348, from 2005 to 2010. The prevalence of the CQ susceptible pfcrt genotype increased from 35% (74/215) to 51% (177/348), respectively before and after 2004 (p<10-3). It tended to increase from 1996 to 2010 (Trend test, p=0.02). Mean estimated 50% inhibitory concentration (IC50) for CQ was 127nmol/L (95% confidence interval [CI], 105 to 150) in 1996-2004 versus 83nmol/L (95% CI, 71 to 94) in 2005-2010 (p<10-3) and the IC50 isolate/Pf3D7 ratio was 5.64 (95% CI, 4.49 to 6.81) (threshold for resistance in this laboratory = 3) versus 2.90 (95% CI, 2.38 to 3.43) (p<10-3), respectively. Thus, a reduction in resistance to CQ following the official withdrawal in 2003 was observed in imported cases from Senegal.

A return of the CQ susceptibility is consistent with observations in Malawi, even if the studied period after CQ withdrawal, was shorter in Senegal than in Malawi (7 years versus 12 years).

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CHLOROQUINE RESISTANCE IN HAITI: LESSONS LEARNED FROM IMPORTED CASES

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On 12 January 2010, an 7.0 magnitude earthquake placed many displaced residents and emergency responders at substantial risk for malaria In Haiti. In the following weeks, US military personal engaged in the relief operations were hospitalised with *P. falciparum* malaria resistant to chloroquine (CQ), first-line treatment for uncomplicated malaria on the island. We investigate if malarial drug resistance profiles (genotypic and phenotypic) of P.f strains detected in imported malaria cases from Haiti could have raised an early warning of chloroquine-resistance prior to this catastrophe. Retrospective data collected in 1988-2010 from malaria surveillance centres in France and in Toronto were studied. *In vitro* response of reference and clinical isolates to CQ and the pfcrt 76T molecular marker for CQ susceptibility were studied in patients with recent travel history in Haiti. In total, 40 P.f isolates were obtained from clinical cases imported from Haiti. Among 3 Canadian clinical isolates, all were pfCRT K76 (wild-type genotype) but after *in vitro* adaptation of two, mutant pfCRT 76T was found. The 50% inhibitory concentrations (IC50) were high for both, 506nM and 708nM. The ratio IC50 isolate/Pf3D7 (a CQ susceptible clone) was respectively 19.5 and 27.2. Among 37 French clinical isolates, all were pfCRT K76 and 29 were analysed ex vivo with a mean IC50 for CQ of 27nM (95% confidence interval [CI], 13.5 to 43.4) and a mean of 3D7 ratio of 1.05 (95% CI, 0.58 to 1.74). Three and 16 patients in Canada and France, respectively, were infected during and after the earthquake in Haiti. Although the few cases observed among travellers are probably not a representative sample, we did not detect early sign of resistance of CQ in Haiti but mixed population of parasites in 2 Canadian samples. It is likely that resistant parasites circulate within a majority of susceptible isolates and that the earthquake created the necessary epidemiological conditions, i.e. population movement, inappropriate shelters, which have contributed to evidence the resistance.

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PERCEPTION, KNOWLEDGE AND PRACTICES ON MALARIA OF THE CONGOLESE POPULATION AND ITS WILLINGNESS TO PARTICIPATE IN CLINICAL TRIALS

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In Republic of Congo, malaria remains a major public health problem. Interventions like bed nets and free treatment with artemisinin-based combination therapies (ACTs) have been introduced in the country without exploring the social dimensions. This is the first pilot study to investigate the perception, knowledge and practices of the Congolese population on malaria and to evaluate its willingness to participate in clinical trials. One hundred informants (65 men, 35 Female) aged 18 to 63 years in Brazzaville were randomly selected on the sampling frame of 2007 census. Sixty five percent and 82% of respondents reached the secondary school education and were economically active, respectively. About two thirds of informants (60%) identified the mosquito as the causative agent of malaria and 47% indicated cleaning the environment for a successful fight against malaria. More than 80% of respondents knew that the health center or hospital was the place for appropriate treatment of malaria while 21% were in favor of self-medication for sick children before consulting a clinician. Sixty-two percent (62%) gave a clear definition of a clinical trial. Willingness to participate in clinical trials was low for vaccines (37%) and higher (62%) for drugs. There was a positive relationship between level of education and "to have heard about clinical trial" and also between level of education and the trust in respect of the confidentiality of data collected by the investigators. In conclusion, the study shows that even though no clinical trial has been carried out in Congo-Brazzaville, the population of Brazzaville was willing to participate in malaria drug trial. Media like TV and radio may have played an important role to increase knowledge about malaria and clinical trial. Issues on risks to participate in clinical trials that needs to be carefully considered by investigators designing clinical trials were highlighted by the population.

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MALARIA ACTIVE CASE SURVEILLANCE: THE CASE FOR LUSAKA URBAN HEALTH FACILITY PROFILING

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Malaria parasite prevalence in Lusaka District, Zambia is extremely low, and confirmed cases are minimal. In response, Zambia's National Malaria Control Program (NMCP) is transitioning its Lusaka intervention strategy from universal IRS and ITN coverage, to more targeted, focal interventions. Among the interventions will be community-based, active infection detection, wherein response teams screen-and-treat neighborhoods of passively detected cases. This is a critical transition point and success will largely depend upon surveillance. Timely, accurate data are necessary to identify infection foci and to quantify the relative impact of this new focal strategy against traditional universal coverage. In order to prepare baseline data to monitor impact, the NMCP collected retrospective malaria data from all 26 urban health centers back to 2004. Surveillance teams visited each clinic and coordinated with the clinic in-charge and data personnel to review clinic and laboratory registers. These data complement the Health Management Information System which captures routine data ranging from disease to service delivery information for clinics and hospitals in all districts. Data elements collected include: total consultations, malaria cases, total tested by RDT and microscopy, total positive by method and the stocks of ACTs dispensed from the pharmacy. A preliminary review of these data has shown interesting trends: although there is very low level of malaria transmission in the district, there remains a relatively high usage of ACTs -- individuals with fevers are often presumptively

treated versus parasitologically confirmed. These data continue to be collected prospectively, on a monthly basis for consistent monitoring of intervention strategies. The profiling exercise provided reliable baseline data for monitoring the future impact of Lusaka District interventions. It also revealed some trends e.g. over-prescription of ACTs, that may warrant additional facility-based interventions. Both points underscore the critical need for accurate and timely surveillance data, particularly as Lusaka District continues towards malaria elimination. The facility profiling exercise has proven a useful method to monitor the successes of surveillance and intervention enhancements, as well as redirect efforts in a data-driven manner, especially during the rollout of malaria active infection detection.

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REACTIVE CASE DETECTION IN A RURAL AREA IN ZAMBIA - YEAR 2 OF TARGETING ASYMPTOMATIC RESERVOIRS OF *PLASMODIUM FALCIPARUM* MALARIA DURING THE LOW TRANSMISSION SEASON

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Asymptomatic foci of malaria may be important reservoirs of parasites and are difficult to detect. A targeted active case detection system using symptomatic cases that present at rural health centres during the low transmission season in the Choma and Namwala districts was conducted in 2009. Results of this pilot study indicated that this system appears to be able to identify more cases of asymptomatic asexual parasite and gametocytes carriers than controls. This study was repeated from July to November 2010 with an increased sample size to further validate the findings. The residence of all rapid diagnostic test (RDT) confirmed cases of malaria from participating rural health centres (RHC) during the low transmission season were located. Two controls per case were selected from the registries of the same RHC on the same day where the case presented. All consenting residents of the selected homesteads completed a questionnaire and were screened for malaria using microscopy, RDT, and nested-PCR. RT-PCR was conducted on all PCR positive samples to detect sexual stage parasites. In total, 218 and 408 participants residing in 40 case and 70 control homesteads, respectively, were screened. Unadjusted analysis resulted in case homesteads having 1.4% prevalence of malaria by RDT and 1.2% in control homesteads (Fisher's Exact p-value = 1.0; OR=1.11 95% CI=0.17-5.76). However, when data were stratified by proximity to permanent rivers, those living greater than 1km away from a river had 2.2 greater odds of being RDT positive for malaria compared to controls (95% CI: 0.29-16.74; Fisher's Exact p-value=0.38). The odds ratio for those living within 1km of rivers could not be calculated but the Fisher's Exact p-value was 0.51. The molecular analysis is ongoing.

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SYSTEMATIC REVIEW OF ANTIMALARIAL MASS DRUG ADMINISTRATION AS A TOOL TO REDUCE MALARIA BURDEN AND TRANSMISSION

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Mass drug administration (MDA), empiric administration of a therapeutic antimalarial regimen to an entire population or well-defined sub-population at the same time, has been an historic component of many malaria control and elimination programs, but is not currently recommended. This strategy is now being re-considered, but data on MDA effectiveness is lacking. We conducted a systematic literature review on the impact of MDA on the incidence or prevalence of asexual parasitemia, gametocytemia, anemia, clinical illness, and mortality. We identified MDA studies in Cochrane Infectious Disease Group Specialized Register, Cochrane Central Register of Controlled Trials (CENTRAL), MEDLINE+, EMBASE, CABS Abstracts, LILACS, and recent conference proceedings. Two authors independently assessed each study for inclusion and abstracted relevant data. Of 2,617 studies identified, 33 met inclusion criteria: 24 before-and-after observational studies, 3 cluster-randomized controlled studies, 3 non-randomized controlled studies, and 3 controlled before-and-after studies. Study dates ranged from 1935 to 2008, and study region ranged from the Americas (6), to Asia (8) and Africa (19). Few studies were conducted in settings with baseline asymptomatic asexual parasitemia rates (PR) ≤5% (5) compared to settings with PR >5% (28). MDA treatment regimens varied: 4 studies included an artemisinin derivative and 11 studies included an 8-aminoquinoline. Following MDA, 24 studies measured asymptomatic malaria parasitemia prevalence, 13 parasitemia incidence, 3 anemia prevalence, 11 gametocytemia prevalence, 1 gametocytemia incidence, 4 mortality, and 12 MDA-associated adverse events. MDA alone was used in 15 studies and combined with other malaria control interventions in 19 studies. Impact results are currently pending. Although we identified many MDA studies, few were well-designed and rigorously conducted. Heterogeneity in study setting, antimalarial regimen, malaria co-intervention use and outcome assessed, hinders making policy decisions based on the current database. Further MDA studies are needed to better understand this malaria control strategy especially with the use of artemisinin-based combination therapies and 8-aminoquinolines.

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APPROACHING MALARIA ELIMINATION IN SWAZILAND: USE OF A PCR-BASED POOLING STRATEGY AND SEROLOGY IN A NATIONAL CROSS-SECTIONAL SURVEY

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Global interest in malaria elimination is high. To guide efforts, countries need accurate assessments of transmission. In low transmission settings, use of pooled polymerase chain reaction (PCR) testing has potential to improve sensitivity and efficiency, and serological data may clarify temporal and spatial trends. Using a stratified two-stage cluster design, a cross-sectional household Malaria Indicator Survey was conducted in 2010 in the malaria endemic region of Swaziland. Blood was collected by finger prick for rapid diagnostic testing (RDT) and on filter paper for pooled PCR and ELISA detecting antibodies to merozoite surface protein-1 ($MSP\text{-}1_{42}$) and apical membrane antigen-1 (AMA-1). Three of 4330 participants tested positive by RDT but negative by PCR. By pooled PCR, one *Plasmodium falciparum* and one *P. malariae* infection were identified among 4031 RDT-negative participants. The *P. falciparum*-infected participant reported recent travel to Mozambique. Compared to performing individual testing, PCR pooling reduced labor and consumable costs by 95·5%. Compared to older participants, seroprevalence among subjects less than 20 years of age was significantly lower (1·9% *vs* 11·7%, p<0.001). Seropositivity was associated with recent travel to Mozambique (OR 4·4, 95% CI 1·0 to 19·0, p=0.048) and residence in southeast Swaziland (RR 3·78, p<0·001). Low overall parasite prevalence and low seroprevalence in younger participants

suggests that recent interventions have been effective and that elimination for this sub-Saharan African country may be feasible. Future efforts should aim to prevent imported malaria, and investigate and target interventions to limited foci of transmission. Pooled PCR and ELISA should be considered in other low transmission settings where accurate surveillance is needed to guide and measure progress of elimination efforts.

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A SPATIAL DECISION SUPPORT SYSTEM FOR MALARIA ELIMINATION INTERVENTION MANAGEMENT

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Regional partnerships have been established to conduct Geographical Reconnaissance (GR) to define the spatial distribution of target populations for malaria elimination in selected provinces of the Solomon Islands (Temotu and Isabel Provinces) and Vanuatu (Tafea Province). The aim was to support long lasting insecticidal net (LLIN) distribution and focal indoor residual spraying (IRS) interventions. GR surveys were carried out using integrated personal digital assistant (PDA) / global positioning system (GPS) handheld units to rapidly map and enumerate households, and collect associated population and household structure data to support long lasting insecticidal net (LLIN) distribution and focal indoor residual spraying (IRS) interventions. Data were uploaded and analysed in customized spatial decision support systems (SDSS) to produce household distribution maps and generate summary information. Subsequent LLIN distribution and focal IRS interventions were coordinated in the three elimination provinces using the SDSS. Following completion of field operations, group discussions were conducted to review and evaluate the SDSS. 16,869 households were geo-referenced and mapped, with a population of 73,664, and 43,714 household structures were recorded. Overall, IRS and LLIN household coverage in target areas were 91.7% and 97.5% respectively. The SDSS provided a strategic tool for coordinating follow-up operations to maximise intervention coverage. An overall high acceptability of the SDSS approach for management of malaria elimination activities was reported. Regional geo-spatial approaches developed for data collection and frontline intervention management have provided an effective operational tool to support the scaled-up demands of malaria elimination in resource-poor Pacific Islands.

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TACKLING MALARIA FROM THE MARGINS OF TRANSMISSION THROUGH A CROSS BORDER MALARIA INITIATIVE BETWEEN MOZAMBIQUE, ZIMBABWE, AND SOUTH AFRICA

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The MOZIZA Cross Border Malaria Initiative is a collaborative partnership between Mozambique, Zimbabwe and South Africa to reduce malaria transmission along shared border areas. The MOZIZA region comprises nine districts: four in Mozambique; four in Zimbabwe and one in South Africa with a total catchment population of 2.3 million people. The malaria incidence in the MOZIZA region ranges from 122-393/1000; 27-335/1000 and 1.69/1000 population at risk in the districts of Mozambique; Zimbabwe and South Africa respectively. As Mozambique strives to halt transmission by scaling up interventions to universal coverage, Southern Zimbabwe and South Africa are embarking on malaria elimination campaigns to achieve zero local transmission. Regional collaboration among these nations becomes important to progressively draw the margins of malaria transmission north by coordinating, harmonising and synchronising interventions across country boundaries. The MOZIZA Malaria Initiative is aligned to the Global Malaria Action Plan and key strategies of World Health Organization and the Southern African Development Community, essentially to scale-up intervention coverage for impact; ensure sustained control and progress towards malaria elimination. A baseline study to identify intervention coverage rates for surveillance, case management, vector control and health promotion has been undertaken to identify gaps and demographic information on the population in the MOZIZA region. This paper describes the current malaria epidemiological situation in the MOZIZA districts and presents data on each of the key coverage indicators for universal coverage and makes recommendations where gaps have been identified.

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SPATIAL STRATEGIES FOR MALARIA CONTROL AND ELIMINATION IN AFRICA

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The intrinsic potential for malaria transmission in the absence of interventions varies across spatial scales from local hotspots through to country and continental differences. To assess both the potential for current and future tools to facilitate local elimination and the degree of effort required to sustain elimination within this heterogeneous landscape, we developed a high resolution continental-scale simulation model for malaria transmission in Africa. The model simulates *P. falciparum* transmission between *Anopheles* vectors and human hosts. Seasonality is incorporated via rainfall dependent mosquito dynamics and temperature effects on sporogony and adult mosquito survival. Spatial and seasonal variation in transmission was modelled at a 1x1km resolution by a carrying-capacity for larval habitats determined by climate, topography, land-use, population density and health service spending (as an indicator of development). Parameter estimates were obtained by fitting the model to age- and season-specific MARA prevalence data (~21,000 observations) from 3597 locations prior to wide-scale intervention (1975-2000). A high correlation (>75%) between observed and predicted PfPr2-10 was achieved, with the correlation rising to >85% at spatial scales over 50km. Scaling-up LLIN distribution across the continent from2000 onwards to the RBM goal of 80% coverage resulted in mean PfPr2-10 being approximately halved across Africa, with much greater decreases in low transmission areas and a consequent "shrinking" of the map. Adding yearly IRS resulted in a 2/3 drop in mean PfPr2-10, with local elimination predicted in locations in Kenya, Ethiopia and Southern Africa. However, these interventions were insufficient to eliminate in areas of intense transmission including West and Central Africa. Two novel interventions - a transmission blocking vaccine with high efficacy and coverage and GM mosquitoes with a strong drive system (X-shredders which disrupt the male/female sex ratio) - were successful in driving transmission to near elimination when added to a combination of high LLIN/IRS coverage, although as a single tool neither was sufficient. Further spatial strategies will be discussed.

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OPTIMIZING STRATEGIES FOR MALARIA ELIMINATION BY MATHEMATICAL MODELLING

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Unprecedented efforts are now underway to eliminate malaria. If they are perceived as failing current high levels of funding will probably not be sustained. It is imperative that methods are developed to use the limited data available to design and optimize site-specific, cost-effective elimination programmes. Mathematical modelling can evaluate different strategies much more rapidly than is possible through trial and error in the field and is being used to guide and inform current elimination efforts. We have developed a range of mathematical models to help optimize the use of antimalarial drugs for malaria elimination. Although initially developed for Cambodia, many of the findings apply in a range of transmission settings and are broadly relevant to malaria control programmes worldwide. In particular these models are being used to predict the relative impact of treatment and mass drug administration (MDA). Antimalarials considered include artemisinin combination therapies (ACT), atovaquone-proguanil and primaquine. The spread and impact of artemisinin and atovaquone resistance with different strategies are examined in detail. A variety of modeling approaches is used to maximize the robustness of findings. The various models will be presented and major results to date summarized. Key findings: 1) high coverage with ACT treatment can produce a long-term reduction in malaria but the impact of a single round of MDA is generally only short-term; 2) primaquine added to ACT reduces time to elimination and slows the spread of artemisinin resistance; 3) atovaquone-proguanil has similar efficacy to ACT when used for MDA; 4) low levels of artemisinin and atovaquone resistance have negligible impact on the efficacy of MDA as the cumulative selective pressure is generally lower than that of treatment; 5) parasite prevalence is a better surveillance measure for elimination programmes than numbers of symptomatic cases; 6) combinations of interventions are more effective than high coverage single interventions; and 7) sustained efforts are crucial for successful elimination.

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PARADIGM SHIFT WITH THE PROGRAMMATIC TRANSITION FROM REDUCING MORBIDITY AND MORTALITY TO THE INTERRUPTION OF TRANSMISSION

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During the past 50+ years, malaria control has emphasized the reduction of morbidity and mortality, and has therefore focused on non-immune subjects such as children < 5 or 10 years of age. In contrast, with the transition to an emphasis on the interruption of transmission, the individuals of greatest interest for malaria control are now likely to be older persons with prolonged asymptomatic parasitemias because they have acquired the semi-immune state. Other challenges in this transition are likely to include a need for: greater caution in the interpretation of negative rapid diagnostic tests (RDTs), the development of novel surveillance strategies for infected individuals and changes in the timing of control interventions. False-negative RDTs based on the detection of histidine-rich protein 2 (e.g., ParaCheck) from spontaneous deletion of the subtelomeric *hrp2* gene are likely to become more frequent with the lower multiplicities of infection found with less intense transmission. Because standard surveillance strategies such as prevalence surveys require increasingly large numbers of subjects as prevalence rates decrease below 1-3%, novel alternative surveillance strategies are likely to be necessary as malaria control improves and prevalence decreases. Finally, the timing of most seasonal interventions is during the peak of the transmission season when the numbers of cases and intensity of transmission are greatest. However, in order to interrupt transmission, it will likely be necessary to focus on the nadir of transmission during the dry season rather than its peak during the transmission season. In summary, moving from the reduction of morbidity and mortality to the interruption of transmission will require different conceptual and practical approaches that have not yet been considered by the majority of investigators and malaria control programs.

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A GLOBAL MAP OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY (G6PDD)

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A major challenge to the ambition of malaria elimination is the relapsing liver stage of *Plasmodium vivax*. Primaquine is the only licenced drug that kills these dormant forms, whilst also causing a significant risk of haemolysis in G6PDd individuals. Knowledge of the prevalence of this genetic condition is therefore essential to help maximise safe deployment of this key component of the malaria elimination toolkit. G6PDd-triggered neonatal jaundice is a further clinical and public health burden. Here, we present the first modelled, continuous global map of G6PDd prevalence. Extensive literature searches were conducted to assemble a database of representative community surveys reporting phenotypically diagnosed G6PDd rates. Surveys meeting a set of standardised inclusion criteria, including being spatially identifiable, formed the evidence base informing the sex-specific Bayesian geostatistical model developed to predict the global prevalence of G6PDd. Combined with high-resolution population density maps, estimates of the affected populations were derived. Both prevalence predictions and population estimates are bounded by credible intervals quantifying uncertainty in the predictions. This map could become integrated into the evidence-base informing malaria control and enabling the tailoring of sub-national policy towards primaquine. Two key limitations highlight the direction of future work required in this field. First, inconsistency between surveys due to the range of diagnostics used may introduce variability to the designation of "deficiency". This variability strongly supports calls for a standardised, field-deployable diagnostic kit. Second, interpretation of the map is limited by major knowledge gaps in the relationship between G6PDd types and primaquine sensitivity. To address this and advance our understanding of G6PDd phenotypic spatial variability, this global prevalence map will be supplemented with distribution maps of the underlying common molecular variants.

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REVEALING A *PLASMODIUM* RESERVOIR AMONG ASYMPTOMATIC INHABITANTS ON ZANZIBAR

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Many countries have recently experienced dramatic reductions in the devastating malaria burden due to wide-scale implementation of successful control measures such as rapid diagnostic tests (RDT), artemisinin based combination therapy, impregnated bed-nets and indoor residual spraying. Zanzibar was among the first regions in sub-Saharan Africa to implement these measures on a wide scale and as such

P. falciparum prevalence decreased to below 1%. This information has raised the ambition from malaria control to elimination! However, this does not rule out the presence of a remaining low density reservoir in the community i.e. parasitaemias below the detection limit of microscopy and/or RDT, which might enable continued malaria transmission. Our aim was to possibly reveal and describe such *Plasmodium* reservoir in the Zanzibar community. PCR screening for the presence of *Plasmodium* parasites was performed on 450 randomly selected blood samples collected on filter papers from a community based cross-sectional survey conducted in the North A district on Unguja Island and Micheweni district on Pemba Island in 2009. In this survey only one of 2423 persons was microscopy positive. The samples were pooled 9-by-9 and after Chelex-DNA-extraction possible parasites were detected by a highly sensitive Cytochrome B nested PCR amplification. Positive pools were re-extracted and analyzed individually for species identification. *Plasmodium* DNA was detected in 26/450 samples (5.8%). 19 were *P. falciparum* (4.2%) and the remaining were *P. malariae.* The prevalence was lower in children < 5 years old (0.9%), while it was higher in older children 5 - 14 years old (12.4%). The prevalence was 1.5% and 8.9% in the North A and Micheweni district, respectively. In summary, we revealed a larger community parasite reservoir than expected, especially in older children and on Pemba Island, which may constitute an important source of transmission in Zanzibar. These findings may have critical implications for future attempts to persue malaria elimination.

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NEW TOOLS FOR MALARIA SURVEILLANCE IN CAMBODIA

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In common with many countries, passive reporting of cases presenting at health facilities forms the mainstay of malaria surveillance in Cambodia. Through the national health information system (HIS), malaria case data are compiled monthly and reported at district level. A parallel system of passive case detection through village malaria workers (VMWs) also provides monthly data, although currently these are not included in HIS reports and, as with the HIS, do not capture self- or privately-treated cases. In addition, since 2004, periodic national malaria surveys have provided data on a range of malariometric indicators at community level. Together, these surveillance activities provide relatively robust, nationally representative data to support strategic planning and monitoring and evaluation. However, two recent developments in Cambodia have highlighted the limitations of these systems in terms of providing timely, spatially specific data suitable for facilitating targeted response at the local level. Firstly, evidence of *Plasmodium falciparum* resistance to artemisinin-based drugs has emerged along the Cambodia-Thai border and containing it requires a surveillance system capable of rapidly identifying and responding to the presence of drug-resistant parasites. Secondly, in March 2011, Cambodia launched a new national strategy to eliminate malaria by 2025. The success of this strategy will in part depend on the availability of detailed spatial data for stratification and real-time information on incident cases. To address these new challenges the Cambodian national malaria programme and partners are developing and testing a variety of novel surveillance approaches, including piloting systems to detect and respond to artemisinin resistant cases and new platforms for VMWs and health facility staff to report data by SMS. Parallel activities are also ongoing to enhance existing HIS and VMW reporting systems to provide spatially specific data to support detailed risk stratification. In this paper we provide an overview of these initiatives and review lessons learned so far in their implementation.

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ZANZIBAR - TOWARDS ELIMINATION?

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The Zanzibar Malaria Control Program (ZMCP) has implemented comprehensive, well integrated combined malaria control interventions, free of charge and with high coverage starting 2003. The main components are long lasting insecticidal nets (LLIN) and indoor residual spraying (IRS) against the vectors, rapid diagnostic tests (RDT) and artemisinin-based combination therapies (ACT) in all public health facilities for malaria case management. The ZMCP initiative has become a unique case study for potential malaria elimination from a malaria endemic area in sub-Saharan Africa. We have studied the respective uptake and overall impact of these interventions more closely in two districts of Zanzibar, North A and Micheweni up to 2010. The impact is assessed with regards to different parameters such as incidence of confirmed malaria cases, child mortality as well as community parasite prevalence. Triangulation of data from community based cross-sectional surveys, health facility records and vital statistics provide evidence of sustainable malaria control in Zanzibar to a level equivalent with malaria pre-elimination.

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IS ACTIVE MALARIA CASE DETECTION IN THE COMMUNITY ABLE TO INHIBIT LOW-LEVEL FOCAL MALARIA TRANSMISSION IN ZANZIBAR?

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Intensive malaria control interventions in Zanzibar, including indoor residual spraying, long-lasting insecticidal nets and artemisinin -based combination therapy have resulted in malaria pre elimination phase (prevalence below 1% in 2011). However, a number of transmission foci have been identified after implementation of a clinic-based passive surveillance system to gather weekly malaria notifications. In 2011 Zanzibar introduced a proactive case detection (pACD) effort to screen the population in transmission foci with the aim to find and treat asymptomatic malaria cases and reduce transmission by decreasing the parasite prevalence in the population. Two small geographic areas were selected, Area-1 with high seasonal transmission (64 km2) and Area-2 with sustained perennial transmission (28 km2). In Area-1 the entire population was tested using a HRP2-based rapid diagnostic test (RDT) and assessed for current fever. In Area-2 all children ≤15 yrs were tested using the same procedures. Screening posts were positioned within the targeted villages over a 3-day period in mid-May 2011, just prior to the predicted increase in seasonal transmission. Confirmed malaria cases were treated with artesunate-amodiaquine according to national guidelines. A total of 6,276 (83%) of the targeted population was screened (83.4% in Area-1 and 83.3% in Area-2). Screening in Area-1and -2 yielded 13 and 64 RDT-positive cases, respectively, with a positivity rate of 0.1% among

residents <5 years of age in both areas. Residents older than five years had a positivity rate of 0.4% and 1.9% in Area-1 and -2, respectively. Variation in village-level positivity rates was detected (0.2-0.6% Area-1 and 1.0-2.7% Area-2). Data regarding clinical symptoms are being analyzed. A second screening session is planned in the first week of June 2011. The community participation in Zanzibar's first pACD effort was high and yielded 77 malaria cases outside of a clinic setting. Malaria cases with potential to perpetuate transmission were identified and treated successfully. Effects of the first and second screening activity on reducing malaria incidence in these communities will be carefully monitored through the existing weekly surveillance system.

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MODELING FOR MALARIA ELIMINATION IN A VARIETY OF TRANSMISSION SETTINGS

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A model is developed for planning malaria elimination in a variety of settings, including variation in baseline transmission intensity, seasonality, and vector population ecology and behavior. Rather than single interventions, the effects of combined interventions are studied for their effects alone and in combination with other interventions. Vector control measures such as insecticide-treated nets (ITNs) and indoor residual spraying (IRS) are studied, along with other forms of vector control such as larval control. These vector control measures are combined with potential pre-erythrocytic and transmission-blocking vaccines. In addition, we include the effects of drug treatments and distributions and study different modalities of distribution. Metrics include reductions in EIR, reductions in detected parasitemia, and reductions in true prevalence. Potential effects of vector control interventions depend strongly on vector ecology and behavior, and the distribution of local species can change in response to interventions. In moderate to high transmission settings, vaccine efficacy depends on the extent to which other interventions reduce baseline transmission. We also study the potential impact of improved diagnostics on test and treat drug administration results. Sensitivities of results to system parameters, vector model parameters, disease model parameters, campaign coverage, and basic model assumptions are explored and uncertainties are quantified. The Garki Project is then modeled retrospectively, as it actually happened, and redone with potentially available interventions.

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ELECTRONIC DATA CAPTURE AND REPORTING METHODS FOR INDOOR RESIDUAL SPRAYING (IRS) ACTIVITIES IN ZAMBIA

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Indoor residual spraying (IRS) along with long-lasting insecticide treated nets (LLIN) form the mainstay of vector and subsequently malaria control throughout sub-Saharan Africa. Zambia has invested heavily in IRS over the past decade and now boasts coverage levels in excess of 35% in urban/peri-urban settings contributing to a significant reduction in national malaria parasitemia from 22% in 2006 to 16% in 2010. Historically, IRS field operators recorded each sprayed house, along with a few limited data elements as a single line item on a paper form. Supervisors would then manually aggregate the data before entering it into a spreadsheet for reporting. Each individual spreadsheet would then be methodically cut-and-pasted into a master spreadsheet document. This slow, labor intensive and error-prone system was only able to collect a limited set of data. To address this issue, an electronic data capture solution has been developed and piloted for rapid collection and dissemination of IRS data. In our pilot study, IRS operators are individually equipped with a personal digital assistant (PDA) that guides them through collecting necessary data elements including GPS coordinates for every structure, spray application details, LLIN usage and previous spray history. Validation rules built into the software ensure that only valid data are entered. Supervisors can review these data at the end of each day, ensuring that data are accurate. Datasets are periodically exported for timely reporting to the district/provincial/central level(s). Importantly, there is no aggregation of data allowing field observations to be specifically analyzed. This feature allows rapid identification of areas of low spray coverage requiring additional IRS mop-up operations. This robust and expanded data collection method allows fine spatial mapping of spray activities to ensure that IRS applications are as effective and efficient as possible.

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ADAPTIVE CHANGES IN MALARIA TRANSMISSION DURING SCALED UP INTERVENTIONS IN SOUTHERN ZAMBIA

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As endemic countries scale up vector control and artemisinin-based combination therapy (ACT) interventions against malaria, widespread reductions in disease burden have been observed. A number of countries are now aiming for possible local or regional malaria elimination, including those in southern Africa, which are located towards the natural fringes of transmission. As intervention coverage is never total, and typically prioritizes vulnerable "non-immune" groups, we examined cross-sectional microscopic and sub-microscopic malaria parasite rates in the resident population of a 2000km^2 vicinity around Malaria Institute at Macha, from 2005-2009. Our data showed low-level microscopy-positive asymptomatic carriage spanning across all ages from less than 5 (3%) to over 65 years (2%). PCR screening showed even higher subclinical parasite rates, peaking in age-groups 10-14 (18%), 35-39 (21%) and 60-64 years old (15%). Furthermore, our data point to a temporal shift of patent gametocytaemia to older ages generally less protected by ITNs. Recent entomological surveys from Macha also suggest that the principal local vector, *Anopheles arabiensis*, is changing behaviour to bite during early evening hours, before bed-time. These changing epidemiological features, as the malaria parasite and vector adapt to interventions, will be key to guiding rational targeting for the next step to achieve malaria elimination. With the resilient scourge prevailing in significant segments of resident communities as asymptomatic and often low-grade, sub-microscopic infections, a spectre of possible resurgence may be looming. A detailed understanding of the epidemiological significance of asymptomatic, especially sub-microscopic infections and their role in transmission is imperative, to aid in policy decisions on targeting for intervention in areas approaching pre-elimination.

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FINANCING THE MOVE TOWARDS MALARIA ELIMINATION THROUGH DOMESTIC RESOURCES IN SOUTH AFRICA

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Financing malaria elimination is a recurring commitment that requires long-term sustainability. As South Africa embarks on a malaria elimination campaign, mobilising resources has become a key priority to operationalise

scale-up of new activities and strengthening of existing interventions. After a comprehensive malaria programme review in 2009, followed by a 10 year retrospective epidemiological analysis of all available malaria information, South Africa's National Department of Health determined that the nation was ready to embark on a national malaria elimination campaign. Subsequently, a strategic plan for malaria elimination (2011-2018) was developed and appropriate interventions were determined over the eight-year timeline. Currently, domestic (government) funding accounts for nearly 99% of all malaria programmatic funding. To ensure sustainability of the malaria elimination campaign, additional government resources will be needed to bridge the funding gap between current control programmes and future elimination programmes. A comprehensive costing exercise of the malaria elimination strategic plan was undertaken and determined that South Africa will need to invest approximately 16 million USD annually above its current budget to completely fund the campaign. In addition to costing the malaria elimination strategic plan, large-scale expenditure reviews of current spending were conducted to determine if any money in current budgets could be re-programmed for elimination activities. A funding gap of approximately 10 million USD annually still persists and exploration of domestic resources to fill the funding gap is underway. This paper presents data on past and present expenditure within malaria control programmes and discusses and makes recommendations on how South Africa can domestically fund its malaria elimination campaign.

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COMPARATIVE ACQUISITION OF ANTIBODIES TO *PLASMODIUM FALCIPARUM* AMA 1 AND MSP 1-19 AMONG CHILDREN LIVING IN TANGA, NORTHEASTERN TANZANIA

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Despite the presently declining trend in most parts of the world, malaria still ranks high among causes of morbidity and mortality in the developing world especially sub-Saharan Africa. Naturally acquired immunity develops as a function of age and exposure to *Plasmodium falciparum* antigens and is in part, antibody mediated. Studies for comparative antibody acquisition to validate putative vaccine candidates are necessary and usually precede longitudinal studies and clinical trials of selected and further developed malaria vaccine candidate molecules. A cohort study was conducted in children aged between 6 months and 10 years in Tanga, Tanzania. A baseline cross-sectional survey was conducted at the beginning of the study. Indirect Enzyme-Linked Immunosorbent assay (ELISA) was performed on collected blood samples to determine comparative natural acquisition of antibodies against malaria-specific antigens AMA-1 versus MSP1-19. Anti-AMA1 IgG, IgG1, and IgG2 levels were significantly higher compared to anti MSP 1-19 levels ($p<0.001$) and the proportion of responders to AMA-1 was higher than anti MSP 1-19 ($p < 0.05$ for total IgG, IgG2, IgG3 and IgG4). Generally, antibody responses were found to increase with age. Natural acquisition of antibodies to malaria antigens increase with age and the levels are dependent on the antigenic stimulation. Low response to MSP 1-19 could be due to declining malaria transmission in the area. Further analysis of the cohort follow-up will be done later to establish how naturally acquired antibodies may protect against malaria-related morbidity. In this era of declining malaria, further research is needed to address malaria morbidity and immunity aspects and more efforts should be directed towards elimination of malaria through integrated approach to malaria management.

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LONG-TERM PROTECTION AND SUSTAINABILITY OF IMMUNOLOGICAL MEMORY INDUCED BY EARLY AND LATE ARRESTING *PLASMODIUM BERGHEI* SPOROZOITES

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To date, inoculation of whole live parasite with differential cycle stage arrest are used in malaria immunization studies. The concept of radiation attenuated sporozoites (RAS) is based on broad disruption of genes with premature arrest of liver stage development. Alternatively, sporozoites administered concomitantly with chloroquine chemoprophylaxis (CPS) undergo full liver stage and develop into blood-stage parasites that are killed by chloroquine. Despite differences in degree of pre-erythrocytic development, both RAS and CPS immunization strategies induced complete protection when applied in mouse and man. Initial studies in the *P. berghei* murine model showed approximately a month after immunization similar protective efficacy and an important role of CD8+ effector memory T-cell response in both protocols. We therefore aimed in this study to investigate the sustainability of the protective immune responses by RAS or CPS. For this purpose, C57BL/6j mice are immunized and challenged after three or six months. At various time-points around challenge, cellular and humoral responses are to be assessed in blood, liver and spleen. Results to be presented comprise: (i) CD4+ and CD8+ T-cell memory, (ii) dynamics of regulatory T cells, (iii) antibody levels and (iv) functionality of T-cell memory response assessed by means of ex vivo assays. Presented data will be discussed in the context of long term protection as one of the main goal in malaria vaccine development.

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HIGH AFFINITY ANTIBODIES TO *PLASMODIUM FALCIPARUM* MEROZOITE ANTIGENS ARE ASSOCIATED WITH PROTECTION FROM MALARIA

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Antibodies are known to be of importance in protection against malaria. In this study, Surface Plasmon Resonance was used to evaluate the affinity of naturally acquired antibodies against *Plasmodium falciparum* merozoite antigens. The antibodies in serum samples from residents of Tanzania, Papua New Guinea, and Uganda bound with different affinities to different antigens. Monoclonal antibodies were also examined. The antibodies to AMA1 were of consistently higher affinity than antibodies to MSP2 antigens. High affinity antibodies correlated with reduced risk of febrile malaria during follow up, and the individuals with the highest affinities had a prolonged time to new infection. We also found indications that different parasites might vary in their ability to induce protective immune responses depending on the specific allele of polymorphic antigen they express. This is important information for understanding how immunity against malaria arises, and for evaluation of malaria vaccine formulations.

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A NANOPARTICLE VACCINE TARGETING *PLASMODIUM FALCIPARUM* CIRCUMSPOROZOITE PROTEIN CONFERS PROTECTIVE HUMORAL AND CELLULAR IMMUNITY

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An ideal vaccine against malaria would be a single platform that could induce long lasting cellular and humoral immune responses without the addition of adjuvant. We have developed a protective vaccine platform for the delivery of *Plasmodium falciparum* circumsporozoite protein (PfCSP) epitopes using an ordered array of NANP peptides displayed on the surface of a self-adjuvanting, self-assembling polypeptide nanoparticle (SAPN). In addition to this B-lymphocyte peptide, there are three different HLA (A2.1, B7 and B35) haplotype selected PfCSP CD8+ T-cell epitopes and a universal CD4+ T-helper epitope, PADRE, included in the SAPN construct. The nanoparticle vaccine can be given in saline either i.p., i.m. or i.v. Here we present our findings of immune modulation and protection against the human malaria *P. falciparum* specific protein in a murine model using a transgenic *P. berghei* sporozoite that expresses the complete *P. falciparum* CSP protein on its surface. Our *P. falciparum* SAPN are able to induce a > 95% protective humoral response for more than 9 months post vaccination with antibodies destroying sporozoites by the classical pathway of complement mediated lysis. Moreover, we are able to induce epitope-specific long-lived memory CD8+ T-lymphocytes which home to and reside in the liver and spleen and, independent of antibody, induce sterile protection against challenge. Our findings present, for the first time, a single platform pre-erythrocytic vaccine capable of inducing long lasting epitope specific protective humoral and cellular immune responses against the human malaria parasite *P. falciparum* CS protein.

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LEVELS OF ANTIBODIES AGAINST *PLASMODIUM FALCIPARUM* MALARIA IN MALIAN CHILDREN ARE MAINTAINED DURING DRY SEASON REGARDLESS OF HEMOGLOBINOPATHY

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Previous studies have shown that children with sickle-cell trait (HbAS heterozygotes) experience fewer *P. falciparum* malaria episodes than children with normal hemoglobin (HbAA homozygotes). To uncover the immunologic differences between these two groups, we initiated a 5-year longitudinal cohort study in Mali where malaria transmission is seasonal and intense. We collected plasmas from children aged 3-12 years in May 2009 (end of the dry season), December 2009 (end of the transmission season) and May, 2010. From 64 HbAS and 61 HbAA children, we collected plasma samples at all three points and determined IgG titers against 4 erythrocytic-stage antigens (AMA1, MSP1, EBA175, and MSP2) by ELISA. HbAS children experienced significantly fewer malaria episodes than HbAA children during the 2009 transmission season, despite showing significantly lower titers than HbAA children in May 2009. These titers increased in both groups of children during the 2009 transmission season and similar differences in titers were found in December 2009. There were no differences in the increase in titers between HbAS and HbAA children and the number of episodes during the transmission season did not correlate with the increase. To explain the lower titers in HbAS children, we determined whether levels of antibodies in HbAS children decay more rapidly compared to HbAA children during the 2010 dry season. Surprisingly, neither HbAA nor HbAS children showed significant reductions in titers from December 2009 to May 2010, despite virtual lack of malaria episodes during this time. In addition, for each of 4 antigens, IgG levels measured in May 2009, December 2009 and May 2010 correlated significantly: i.e., children with higher titers in May 2009 showed higher titers in December 2009 that were maintained until May 2010. These results indicate that IgG titers are maintained in Malian children for 5 months through the dry season, and suggest that IgG levels may be determined mainly by host and/or environmental factor(s) that do not change over relatively short periods of time.

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EVOLUTION OF MULTI-DOMAIN STRUCTURES IN MALARIA PARASITE ANTIGENS

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The *Plasmodium falciparum* var gene family encodes the surface expressed virulence factors PfEMP1, which mediate cytoadherence of infected red blood cells to a variety of host cell receptors. These diverse antigens are also one of the main immune targets, and protection against clinical infection has been shown to correlate with the acquisition of a repertoire of antibodies specific to different PfEMP1 variants. Var genes are characterized by a modular structure, and encode between two and nine different binding domains exhibiting varying binding specificities. In traditional theoretical models of genetically diverse pathogen populations, strong cross-immunity is expected to select against pathogen strains expressing antigens with multiple immunogenic epitopes. Given their highly immunogenic properties and the general abundance of target receptors it is not clear, therefore, why var genes encode multiple binding sites at once. Here, we show that models incorporating antibodies that function to prevent binding rather than preventing infection per se can lead to the evolution of antigens with multiple domains. Under this framework, the acquisition of an additional binding domain can relax the functional constraints upon the first domain, leading to antigenic diversification at that locus without compromising cell adhesion. Under these circumstances we predict that var genes would evolve towards multi-domain structures in which high affinity binding domains would associate with other low-affinity but antigenically diverse domains. We test this prediction by analyzing the domain structures of var genes from published *P. falciparum* genomes, and show that groups of var genes previously classified according to promoter regions A, B, and C, have distinct structures in this regard, with domain diversity varying significantly across the length of long var genes. Our approach represents the first attempt to explain the evolution of multi-domain structures among these antigens, and we show how domain-specific recognition is expected to develop with age in endemic regions of varying transmission intensity.

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ASSESSING THE REACTIVITY OF ANTIBODIES IN MALIAN CHILDREN AGAINST VARIABLE SURFACE ANTIGENS ON *PLASMODIUM FALCIPARUM* INFECTED RED BLOOD CELLS USING A FLOW CYTOMETRY-BASED ASSAY

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To profile the development of immune responses to *Plasmodium falciparum* antigens, we have conducted a longitudinal cohort study of children in Mali where malaria transmission is seasonal. As part of this study, we collected plasma from Malian children aged 3-11 years (total of 175 age- and hemoglobinopathies-matched) at the start (May) and end (December) of the 2009 transmission season. As reported in other studies, children with sickle cell trait (HbAS) experienced significantly fewer malaria episodes compared to children with normal HbAA. Previously, we evaluated plasma IgG titers against merozoite antigens by ELISA and found that HbAS children had significantly lower IgG titers compared to HbAA children. Using the same plasma samples, we compared the levels of antibodies to surface antigens on *P. falciparum* trophozoite-infected RBCs (iRBCs). We devised a novel high-throughput flow cytometry assay, based in part on an immunofluorescence microscopy assay used by Blythe et al. (Infect. Immun. 2008) to assess the reactivity of antibodies to surface antigens on iRBC. First, we wanted to evaluate whether age, sex, and hemoglobinopathies influence acquisition of antibodies to the iRBC surface. We found that some children produced antibodies to FCR3-iRBC. Older children tended to have higher titers to FCR3-iRBC, but these results were not statistically significant. Across all age groups, there was significantly higher titers to FCR3-iRBC at the end versus the start of the transmission season ($P<0.003$), suggesting that children were acquiring new and/or boosting existing IgG responses to iRBCs. Notably, although HbAS is associated with reduced levels of PfEMP-1 (a major variant surface antigen) on the surface of iRBCs, there was no significant difference in IgG titer to FCR3-iRBCs between HbAA and HbAS children at the start or end of the transmission season. We are also determining whether antibodies from Malian children recognize parasites from different geographic regions so that responses to other *P. falciparum* isolates will be gauged.

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ANTIBODY RESPONSES TO SELECT MALARIA ANTIGENS DIFFERENTIALLY DEVELOP AND WANE BY MALARIA TRANSMISSION INTENSITY

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The development of anti-malarial antibodies that mediate protective immunity to *Plasmodium falciparum* (Pf) infection depend on malaria transmission intensity. However, more information is needed on the heterogeneity and kinetics of this multi-antigen response, particularly in areas of unstable malaria transmission. A cohort of 236 children aged 10 months - 15 years, living in areas of stable (Kisumu) and unstable (Nandi) Pf-malaria transmission in Kenya, were surveyed at baseline and six-months later. Determinants of IgG responses to five *P. falciparum* antigens (AMA1 3D7, AMA1 FVO, MSP1 3D7, MSP1 FVO, and LSA1) were contrasted between the two areas. Comparisons in the relative change of antibody responses between the 6-month interval were also conducted. The proportion of positive IgG responses for all age groups was higher in Kisumu than Nandi; these were significant ($P<.05$) for AMA-1 3D7, AMA-1 FVO, and LSA-1. Antibody responses increased with age in Nandi but varied in Kisumu. Children 0-4 years old in Nandi had a two-fold difference ($P<.05$) in the median relative change in IgG responses to AMA-1 3D7, AMA-1 FVO and MSP-1 3D7 over a six-month period than similarly aged children in Kisumu. Antibody responses to AMA-1 3D7, AMA-1 FVO, MSP1-3D7, and LSA-1 among aparasitemic children were higher ($P<.05$) in Kisumu than Nandi. There were differences ($P<.05$) in antibody responses by parasitemia status in Nandi but few in Kisumu. Males and females in Kisumu had higher ($P<.05$) antibody responses to AMA-1 3D7, AMA-1 FVO, MSP1-3D7, and LSA-1 than those in Nandi. All measured antibodies correlated strongly with one another in Nandi ($P<.001$) but few correlated in Kisumu. Important differences in the dynamics and duration of naturally acquired immunity to *P. falciparum* exist by age, parasitemia status, and sex between areas of stable and unstable malaria transmission. These findings highlight the need to consider multiple factors beyond simply which antigens to target for vaccine development.

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CHARACTERIZATION OF THE HUMAN CD4 T CELL RESPONSE TO *PLASMODIUM FALCIPARUM* MALARIA

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In endemic areas clinical immunity to malaria can be acquired but only after repeated *Plasmodium falciparum* infections. Antibodies are known to play a major role in acquired immunity to malaria. Recent studies suggest that the gradual acquisition of protective humoral immunity to malaria may be due in part to the relatively inefficient acquisition of *P. falciparum*-specific memory B cells and long-lived plasmas cells. CD4 T helper cells play a critical role in orchestrating effective B cell responses in the germinal center - from B cell activation, affinity maturation and Ig class switching to memory B cell and plasma cell differentiation. However, little is known about the magnitude, quality, and antigen specificity of the CD4 T cell response to *P. falciparum* infection. In this study we stimulated PBMCs from malaria-experienced donors from Mali with lysates of different blood stages of *P. falciparum* (strain 3D7). After 18 hours of co-culture the intracellular production of IFNγ, TNFα, IL-2, IL-4 and IL-10 was measured in T cell subsets by flow cytometry. Importantly, given their role in supporting B cell responses in the germinal center reaction, we focused in particular on the phenotypic and functional analysis of follicular T helper cells (T_{FH}), identified by the expression of the transcription factor Bcl-6 and several chemokine receptors and activation markers. Comparing the CD4 T cell response, and the T_{FH} cell compartment in particular, of malaria-naïve and experienced persons of different ages and to different stages of the *Pf* life cycle may provide valuable insights into the mechanisms underlying the delayed acquisition of immunity to malaria.

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QUANTIFYING THE IMPORTANCE OF ANTIBODIES TO PFEMP1 AND OTHER SURFACE ANTIGENS OF *PLASMODIUM FALCIPARUM*-INFECTED ERYTHROCYTES, AND THEIR ROLE AS TARGETS OF PROTECTIVE IMMUNITY

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Effective clinical immunity that protects against symptomatic malaria in humans develops gradually after repeated exposure to *Plasmodium falciparum*. However, the primary targets and mechanisms of immunity are not well understood. Upon invasion of host erythrocytes, *P. falciparum* dramatically remodels the host cell for its own survival advantage via the export of novel parasite proteins. These modifications include the expression of variant surface antigens (VSA) on infected erythrocytes (IEs), which include PfEMP1 (*P. falciparum* erythrocyte membrane protein 1), Rifin, STEVOR and SURFIN proteins encoded by multigene families. Antibodies to VSAs are variant-specific and are associated with protection from symptomatic and severe malaria. However, the significance of each of these VSAs as targets of acquired immunity remains unclear due to a lack of tools to quantify antigen-specific responses. In this study, we used novel assays to dissect the importance of PfEMP1 and other VSAs as antibody targets using mutant parasite lines in which surface expression of PfEMP1 was inhibited with transgenic approaches. Comparisons between antibody reactivity to IEs of parental versus mutant parasites allowed us to quantify the importance of PfEMP1 and other VSAs as immune targets. This approach was applied to longitudinal cohorts of adults and children in Kenya and Papua New Guinea (PNG). Results from both populations indicate that PfEMP1 is the major target of naturally acquired antibodies to surface antigens of IEs, and that antibodies specific to PfEMP1 are associated with protective immunity. Antibodies to VSAs are thought to act by promoting phagocytic clearance of IEs and we further demonstrated that PfEMP1 is the major target of antibodies that mediate opsonic phagocytosis. A subset of individuals had prominent antibodies to VSAs other than PfEMP1, suggesting that other VSAs may still have an important role as immune targets. These findings are invaluable to understanding acquired immunity to malaria and advancing vaccine development.

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APICAL MEMBRANE ANTIGEN 1 IS A MAJOR TARGET OF HUMAN INVASION-INHIBITORY ANTIBODIES AGAINST *PLASMODIUM FALCIPARUM* MEROZOITES

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Acquired human immunity to *Plasmodium falciparum* malaria is thought to be mediated in part by antibodies to merozoite antigens that act by inhibiting erythrocyte invasion and blood-stage. Knowledge of the key targets of these antibodies and significance of polymorphisms in antigens will greatly benefit vaccine development. However, the major targets of protective and invasion-inhibitory antibodies in humans remain unclear. Apical membrane antigen 1 (AMA1) is an essential erythrocyte invasion ligand and leading vaccine candidate. Although studies have shown that antibodies to AMA1 are associated with protective immunity, there is little data on the functional activity of antibodies. We investigated the importance of AMA1 as a target of acquired inhibitory antibodies and the significance of AMA1 polymorphisms in a cohort of children and adults in Papua New Guinea. We generated transgenic *P. falciparum* lines expressing six different AMA1 alleles on the same genetic background and used these transgenic parasites in novel assays to quantify AMA1-specific invasion-inhibitory antibodies. This approach enabled the detection of AMA1-specific inhibitory antibodies separately from other inhibitory antibodies. We found that AMA1 is a major target of human invasion-inhibitory antibodies in both children and adults. Measuring AMA1 antibodies by standard ELISA and competition ELISA suggests that antibodies to different AMA1 alleles have a similar prevalence in the population. However, the prevalence of allele-specific invasion-inhibitory antibodies varied substantially for different alleles; there was a high prevalence of inhibitory antibodies for some alleles, but a very low prevalence for other alleles. These results have important implications for understanding the targets and acquisition of human immunity and vaccine development, suggesting that specific AMA1 alleles would be preferred over others in a multi-allele AMA1 vaccine.

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ACTIVELY-INDUCED ANTIGEN-SPECIFIC CD8$^+$ T CELLS BY EPITOPE-BEARING PARASITE PRE-INFECTION BUT NOT PRIME/BOOST VIRUS VECTOR VACCINATION COULD AMELIORATE THE COURSE OF *PLASMODIUM YOELII* BLOOD STAGE INFECTION

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Although malaria parasite is an obligatory intracellular microorganism, the lack of MHC molecules on red blood cells had questioned the immunological function of CD8$^+$ T cells against malarial blood stage (MBS) infection. Several recent reports, however, contradicting with this notion, suggested their influential function on the course of MBS infection. In the present study, we generated genetically-engineered murine malaria, *Plasmodium yoelii*, which expresses a well-defined *Trypanosoma cruzi*-derived, H-2K^b-restricted CD8$^+$ T cell epitope, ANYNFTLV. Prime / boost vaccination by the use of recombinant adenovirus and recombinant MVA, which induced enhanced number of antigen-specific CD8$^+$ T cells, failed to prevent pathological outcome to occur in the course of ANYNFTLV-expressing murine MBS infection. In contrast, the pre-infection of mice with *T. cruzi* which intrinsically bears the same CD8$^+$ T cell epitope significantly improved the survival of ANYNFTLV-expressing malaria-infected mice but not that of control malaria-infected ones. We conclude that the actively-induced antigen-specific CD8$^+$ T cells could ameliorate the pathologies caused by the MBS. Although the protection was observed only in certain situation, our study indicated an important clue that the CD8$^+$ T cell-mediated vaccine against MBS would be feasible.

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INDUCTION OF MALARIA IN VOLUNTEERS BY INTRADERMAL INJECTION OF CRYOPRESERVED *PLASMODIUM FALCIPARUM* SPOROZOITES

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Vaccines and new drugs are needed to prevent the nearly one million deaths and hundreds of millions of cases caused by malaria annually. To develop immunization strategies and to assess experimental anti-malarial vaccines and drugs, volunteers are immunized by the bites of laboratory-reared, malaria sporozoite (SPZ)-infected mosquitoes, or challenged by such mosquitoes after being immunized with experimental vaccines or treated with experimental drugs. Such studies have been critical for development of the most promising experimental vaccines and vaccine strategies, and of anti-malarial drugs. Because it is technically and logistically challenging to produce infectious, SPZ-infected mosquitoes, only a few laboratories in the world perform such experiments. In this trial we assessed the capacity to infect volunteers by intradermal (ID) injection of aseptic, purified, vialed *Plasmodium falciparum* (Pf) SPZ that had been cryopreserved for more than a year. Eighteen healthy Dutch adult volunteers received ID injections of PfSPZ in an open-label, dose-escalation study. Volunteers (N=6/group) received 2,500, 10,000, or 25,000 PfSPZ. The primary outcome variable was detection of blood-stage parasites by microscopy. Kinetics of blood stage parasitemia were assessed by quantitative PCR. Fifteen of eighteen volunteers (84%) developed Pf parasitemia, 5/6 volunteers from each dose group. There were no differences between groups in time until detectable parasitemia, parasite kinetics, clinical symptoms and signs, or laboratory values. We have demonstrated that aseptic, purified, cryopreserved PfSPZ manufactured in compliance with regulatory standards and administered ID infect volunteers. These PfSPZ can be used to assess the efficacy of new antimalarial vaccines and drugs in any clinical trial center in the world. It was recently demonstrated that immunization of volunteers taking chloroquine by exposure three times to PfSPZ-infected mosquitoes induced 100% protection against Pf infection. The PfSPZ used in our current study can translate this artificial immunization protocol into an implementable vaccine.

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THE USE OF FUNCTIONAL IMMUNOASSAYS (ISI AND ILSDA) BASED ON CRYOPRESERVED PRIMARY HUMAN HEPATOCYTES FOR SCREENING PRE-ERYTHROCYTIC *PLASMODIUM FALCIPARUM* VACCINE CANDIDATE ANTIGENS

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The inhibition of sporozoites invasion (ISI) assay and inhibition of liver stage development assay (ILSDA) were developed to functionally assess the effect of humoral immune response on sporozoite invasion and liver stage development *in vitro*. Previously we reported that cryoperserved primary human hepatocytes (CPHH) provided superior invasion rates for *Plasmodium falciparum* compared to the traditional cell lines (HepG2 and HCO4, respectively) used for these two assays. We also reported the development of a real time PCR (RT-PCR) procedure to detect the malaria parasite infection load. Here we demonstrate the correlation of RT-PCR with fluorescent microscopy results as assay read-outs. In addition, we present data evaluating the ability of polyclonal sera induced in mice and/or rabbits against novel pre-erythrocytic *P. falciparum* candidate vaccine antigens to functionally inhibit sporozoite development using the optimized ISI and ILSDA assays. Our results indicate the value of these assays for use as antigen discovery down-selection tools.

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CAN ANTIGENIC DIVERSITY OF APICAL MEMBRANE ANTIGEN-1 BE OVERCOME? RATIONALE FOR THE DEVELOPMENT OF A SECOND GENERATION AMA1 VACCINE

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Apical Membrane Antigen-1 (AMA1) vaccination induces invasion inhibitory antibodies in both naïve and malaria immune populations, however, its antigenic diversity continues to pose a considerable hurdle in Phase 2 efficacy trials. Analysis of a large number of field isolate sequences show a continuum of variability is present within AMA1 with no clear allele families like those of MSP1 and MSP2. Mapping of antigenic escape residues within AMA1 showed that a small group of polymorphic residues on the C1L loop were the primary determinants of strain-specificity. Confining the diversity analysis to only the C1L genotype made it possible to divide AMA1 alleles into a small number of allelic groups. C1L based grouping was also useful for assessing allele specific protection in humans. We have now expressed and purified AMA1 from seven major C1L groups in the *E. coli* expression system. Using anti-sera generated in rabbits against these seven monovalent vaccines, we selected a set of strains to be included in a future second generation AMA1 vaccine. As a proof of concept for a polyvalent approach to overcoming antigenic diversity, we immunized a group of rabbits with a Quadrivalent AMA1 vaccine composed of 3D7, FVO, HB3 and W2mef strain (Quadvax). The C1L sequence analysis showed that Quadvax was able to cover C1L polymorphisms present in ~95% of the field isolates. Cross-strain GIAs also showed a varying degree of susceptibility of all the *P. falciparum* tested, in particular strains that were not present in the Quadvax. Further analysis of the mechanisms of cross-strain invasion inhibition showed that mixing more than two AMA1 alleles in a vaccine can have synergistic effect on the quality of the induced antibodies. Formulating a polyvalent vaccine such as the Quadvax allows us to increase the molar concentration of conserved epitopes in an AMA1 vaccine, which in turn results in refocusing the antibody response to more conserved regions of the protein outside domain-1. All the evidence so far suggests the need for continued development of AMA1 as a component of a multi-stage malaria vaccine.

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HIGH THROUGHPUT GENOMICS SCREENING FOR MALARIA ANTIGEN DISCOVERY

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Malaria is the most burdensome parasitic disease of man, exacting an estimated toll of 863,000 deaths and 243 million clinical cases per year. It is important to develop a vaccine that can effectively prevent the disease. Up to now, there are few identified malaria antigens, representing less than 0.3% of the 5,300 proteins encoded by the *Plasmodium* parasite, and those have limited efficacy in vaccine clinical development. Here, we report a new approach employing adeno-array technology for high-

throughput discovery of pre-erythrocytic *P. falciparum* antigens using orthologues identified in the *P. yoelii* mouse model.To identify highly expressed *P. yoelii* pre-erythrocytic antigens for the adeno-array, we performed bioinformatics data mining using publicly available genomic and proteomic databases. Based on expression abundance data from microarray and protein mass spectrometry analysis by several research groups, we prioritized 300 sporozoite stage and liver stage candidate *P. yoelii* genes with identifiable *P. falciparum* orthologues for generation of the adeno-array. These genes were cloned into a high-level expression cassette located in the E1 region of an E1/E3-deleted adenovirus type 5 genome using high-throughput methodologies. In the antigen discovery screen, we infected antigen presenting cells (APC) with individual adenovectors from the adeno-array. The infected APC were then incubated with splenocytes from mice immunized with known protective regimens of Radiation Attenuated Sporozoites (RAS), and antigen-specific $CD8^+$ T cell responses were measured by Intracellular Cytokine Staining. So far, we have identified 37 new antigens that recalled antigen-specific CD8+ T cell responses from RAS-immunized mice. We are currently testing these adenovirus vectors from the array for their capacity to protect mice from a *P. yoelii* sporozoite challenge. The most highly protective antigens will be prioritized for malaria vaccine development and their *P. falciparum* orthologues will be cloned into adenovectors and advanced to preclinical testing.

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INDUCING POTENT IMMUNE RESPONSES TO MULTIPLE LIVER-STAGE MALARIA ANTIGENS USING DNA VACCINES

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Despite intense research efforts, the currently most advanced malaria vaccine candidate, RTS,S, an adjuvanted recombinant protein, confers only partial protection against clinical disease. Thus, the development of a vaccine that induces long-term protection against malaria remains an important global goal. We have developed a DNA-based vaccine candidate targeting 4 important *Plasmodium falciparum* (P.f.) liver-stage antigens: circumsporozoite protein (CS), liver stage antigen 1 (LSA1), thrombospondin-related-anonymous-protein (TRAP) and cell-traversal protein for ookinetes and sporozoites (CelTOS). Consensus antigens were designed for each vaccine target with several modifications to improve expression. Immunogenicity of the vaccines and a multi-antigen vaccine cocktail (containing all 4 vaccines), delivered with electroporation (EP), was initially evaluated in mice. The vaccines elicited strong antigen-specific T cell responses that were similar to, or surpassed, those induced by other vector systems. Specifically, the vaccine induced an IFNγ response as measured by ELISpot (SFU): CS (1607±391), LSA1 (1908±821), TRAP (929±255) and CelTOS (477±160). Flow cytometry indicated vaccine-specific CD4+ T cell production of IL-2 (2.5%) and TNFα (1.4%) and CD8+ T cell production of IFNγ (0.9%), IL-2 (3.1%) and TNFα (1.5%). LSA1-specific IFNγ production was also detected in 1.5% of hepatic CD8+ T cells. Both vaccine approaches induced robust antigen-specific serotype conversion (IgG endpoint titers >100,000). Inclusion of IL-28B in the vaccine cocktail increased the total IFNγ response and decreased the regulatory T cell population. An on-going non-human primate (NHP) study is evaluating the immune responses elicited by this promising P.f. vaccine candidate. Results from a pilot study indicate this vaccine, delivered intradermally with IL-28B by EP, induces robust immune responses in NHPs. After the 2nd immunization, endpoint titers for CS and LSA1 were >400,000 and >75,000 for TRAP and CelTOS. The vaccine induced 328.3±129.0 IFNγ SFU following the 2nd vaccination and this response increased 4.6-fold with the 3rd vaccination (1512.3±321.6 SFU). In summary, we have developed a novel DNA-based malaria vaccine that elicits potent immune responses, which exceed or are equivalent to the levels induced by other vaccine platforms, indicating this promising vaccine candidate merits further study in human clinical trials.

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POST-PHASE III EVALUATION OF THE RTS,S MALARIA VACCINE CANDIDATE - THE WAY FORWARD

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The RTS,S malaria vaccine candidate has the potential to play an important role as part of future integrated control programs to further reduce the burden of malaria. It is several years ahead of any other malaria vaccine in terms of assessment of clinical efficacy. Following the Phase II results from Mozambique (30% protection against clinical malaria, and close to 50% protection against severe malaria) Kenya and Tanzania (children 5-17 months old; vaccine efficacy against 1st or only episode was 39% over a 12 months follow-up), a large Phase III pre-licensure trial was initiated; initial results will be available in the last quarter of 2011. If the results from the Phase III trials are similar to those from the Phase II trial, we will have a vaccine that is capable of reducing severe malaria by half. The public health relevance of this vaccine, when used to complement existing malaria control and elimination efforts could be immense. There will be a need for effectiveness studies to rapidly assess the full programmatic impact of the vaccine. The clinical trials investigators and associated partners have started to address this need. 4 main axes of development are discussed. 1) continue clinical trials in special populations such as malnourished and/or HIV infected individuals, 2) careful analysis and in-depth evaluation of the protective immune responses and related modeling to better understand the vaccine's potential mode of action, 3) modeling of the potential effect of vaccination in different endemic settings and as part of different integrated intervention strategies/ intervention mixes including economic appraisals, and 4) contribute to the policy dialogue and process that will guide recommendations for use. Most malaria endemic countries in Africa will be faced with decisions on the integration and routine use of the candidate malaria vaccine, and donors will have to decide on financing its introduction into endemic countries, since the expectation in endemic countries will be that children should be vaccinated at no cost to the family. The malaria community will be faced with the ambitious goal of achieving malaria elimination through an expanded malaria control program that includes vaccination and is tailored to the different socio-ecological endemic settings. The clinical trials investigators and associated partners are willing to actively contribute to this endeavor.

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PHASE III SAFETY EVALUATION OF THE RTS, S/AS01 MALARIA VACCINE CANDIDATE: REACTOGENICITY, UNSOLICITED ADVERSE EVENTS AND FATALITIES

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The RTS, S/AS01E malaria candidate vaccine is currently being evaluated in a multicenter Phase 3 randomized controlled double blind trial, in children aged 5 to 17 months and 6 to 12 weeks old at first vaccination, across 11 African sites in 7 African countries. Acceptable safety profile will be paramount in determining whether the candidate vaccine is suitable for implementation in sub-Saharan Africa future vaccination programs. The following safety results will be presented: solicited local and general reactogenicity within 7 days post vaccination and unsolicited Adverse Events occurring within 30 days post vaccination in a subset of 200 subjects of the 5-17 months old age category from each study centers (N=2200) and Serious Adverse Events (all, fatal, related) in all children (N = 15460) from dose 1 up to the 31 May 2011. Seizures occurring within 7 days post vaccination will be presented as per Brighton collaboration guidelines. All results will be tabulated and presented with 95%

Confidence Interval per treatment arm. All unsolicited Adverse Events will be reported classified by MedDRA preferred term. Safety analyses will be performed on the Intention-To-Treat population. Unsolicited events were captured by passive detection. Access to clinical evaluation and care was facilitated in all study centers.

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DESORPTION OF CONJUGATED PFS25-REPA FROM ALHYDROGEL BY A HIGH PH METHOD

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Pfs25 is an ookinete surface protein of the *Plasmodium falciparum* malaria parasite that elicits transmission-blocking antibodies in animals and humans. Pfs25 has been chemically conjugated to the recombinant non-toxic carrier protein *Pseudomonas aeruginosa* ExoProtein A (rEPA) to increase immunogenicity, and a Pfs25-rEPA malaria vaccine candidate was prepared using Alhydrogel, a commercially available aluminum hydroxide adjuvant. In order to satisfy the regulatory requirements for identity and integrity testing of this formulation, we have developed a method to extract a sufficient amount of Pfs25-rEPA from Alhydrogel for further analysis while maintaining epitope functionality. Two previously developed extraction methods relied on buffers containing high salt concentrations, notably citrate and phosphate, to lower the adsorptive capacity of alum during incubation for extended periods of time, ranging from 2.5 to 5 hours. Testing revealed these extraction buffers removed a less than desirable amount of the Pfs25-rEPA conjugate from this formulation. A third method was developed which involved raising the pH of the formulation above the isoelectric points of Alhydrogel (pI ≈ 11) and the Pfs25-rEPA conjugate (pI ≈ 5.57), and this method eluted the maximal amount of Pfs25-rEPA conjugate from alum. Desorption of the Pfs25-rEPA conjugate was immediate, which may be due to the negatively charged antigen and adjuvant in the formulation. Accordingly, the incubation time was minimal and the entire procedure was completed within minutes. Furthermore, the retention of epitope functionality was demonstrated by Western blot using conformational antibody 4B7. In conclusion, the high pH method allowed the greatest desorption of Pfs25-rEPA conjugate from alum and facilitated identity and integrity evaluations of the Pfs25-rEPA on Alhydrogel malaria vaccine candidate.

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ACCELERATED STABILITY STUDY OF PFS25-EPA CONJUGATES FOR A TRANSMISSION-BLOCKING VACCINE

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Pfs25, the major surface protein of *Plasmodium falciparum* zygotes, is a leading malaria transmission-blocking vaccine candidate. To enhance the immunogenicity of Pfs25, we have conjugated the protein to recombinant nontoxic *Pseudomonas aeruginosa* ExoProtein A (rEPA), and the stability of the conjugate was evaluated in this study. Pfs25-EPA conjugate was subjected to three freeze/thaw cycles, and was also evaluated for thermal stability after storage at temperatures of 4°C or 37°C for 7, 14 and 56 days, and at -80°C for 56 days. The forced freeze/thaw and thermal stability samples were analyzed for identity by Western blot with monoclonal antibodies 4B7 and penta-His, and polyclonal anti-EPA antibodies, and for integrity by Tris-Acetate SDS-PAGE with silver staining, capillary gel electrophoresis (CGE) using pluronic F-127 as resin, and size-exclusion chromatography with multi-angle light scattering (SEC-MALS). Our results showed that the Pfs25-EPA conjugate was stable following three freeze/thaw cycles. The conjugate was also stable for up to 56 days at 4°C or -80°C. However, change was observed in samples stored at 37°C for 7 days or longer, as evidenced by the release of low molecular weight proteins and a decrease in average molecular mass from 558 kDa on day 0 to 367 kDa on day 56. These results indicate that the stability of the Pfs25-EPA conjugate is suitable for human testing.

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TRANSMISSION BLOCKING ASSAYS FOR CLINICAL DEVELOPMENT OF VACCINES TO INTERRUPT MALARIA TRANSMISSION

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Transmission blocking vaccines work by inducing antibodies in vaccinated individuals that inhibit the development of malaria parasites in the midgut of the mosquito, thus interrupting the cycle of transmission to the next human host. A standard membrane feeding assay has been used to evaluate the ex vivo transmission blocking activity of antibodies induced by vaccine candidates, but the assay needs to be qualified to determine to what extent it is predictive of transmission blocking activities in the field. A study is currently under way to compare results of mosquito feeding assays in malaria exposed adults and children in Bancoumana, Mali. Insectary-raised progeny of field-caught mosquitoes are directly fed on gametocytemic individuals and age-matched gametocyte negative individuals. Infectivity in these mosquitoes is then compared against those of mosquitoes fed in direct membrane feeding assays in Mali and standard membrane feeding assays in the USA. Data will also be generated on the dynamics of gametocyte carriage rates through the year. Results to date of feeding experiments will be presented, and potential clinical development paths for transmission blocking vaccines using these data will be discussed.

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PRE-CLINICAL DEVELOPMENT OF A POTENT TRANSMISSION BLOCKING PLANT-PRODUCED *PLASMODIUM FALCIPARUM* SEXUAL STAGE PFS25 VACCINE CANDIDATE

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Malaria is a serious and sometimes fatal mosquito-borne disease caused by a protozoan parasite. Each year it is estimated that over one million people are killed by malaria and yet the disease is preventable and treatable. Developing vaccines against the parasite is a critical component in the fight against malaria and these vaccines can target different stages of the pathogen's life cycle. We are targeting sexual stage proteins of *Plasmodium falciparum* which are found on the surface of the parasite's reproductive cells present in the mosquito gut. Antibodies against these proteins block the progression of the parasite's life cycle in the mosquito, and thus block transmission to the next human host. Transmission blocking vaccines are essential to the malaria eradication program to ease the disease burden at the population level. In the work presented here, we focus on the process development, formulation, scale-up and pre-clinical evaluation of a potent Pfs25 recombinant antigen that shows

effective transmission blocking activity. The antigen was successfully expressed in our plant-based launch-vector system and purified to a high level of homogeneity. The resulting Pfs25 antigen has undergone high throughput screening formulation development, extensive biochemical characterization and dose ranging studies to determine the minimal effective dose in pre-clinical animal studies. The purification process has been successfully scaled through pre-clinical production levels in preparation for large-scale production in our pilot GMP facility. These data demonstrate the feasibility of expressing *Plasmodium* antigens in a plant-based system for the economic production of a transmission blocking vaccine against malaria.

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QUANTITATIVE ASSESSMENT OF *PLASMODIUM FALCIPARUM* SEXUAL DEVELOPMENT REVEALS POTENT TRANSMISSION-BLOCKING ACTIVITY OF THE SYNTHETIC DYE METHYLENE BLUE

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Drugs that block the transmission of *Plasmodium falciparum* sexual stage parasites to mosquito vectors could play a key role in eliminating malaria. However, efforts to measure the activity of existing antimalarials on sexual stage gametocytes and to identify transmission-blocking agents have been hindered by a lack of quantitative assays. Here, we describe experimental approaches using *P. falciparum* GFP-luciferase reporter lines that enable the assessment of dose- and time-dependent drug action on gametocyte maturation and transmission. Our studies reveal activity of the first-line antimalarial dihydroartemisinin and the partner drugs lumefantrine and pyronaridine on immature gametocytes, along with appreciable inhibition of mature gametocyte transmission to *Anopheles* mosquitoes. Prophylactic 8-aminoquinolines had broad gametocytocidal activity at elevated concentrations. In contrast, methylene blue potently inhibited all gametocyte stages and almost fully abolished transmission to mosquitoes at concentrations achievable in humans, highlighting its potential to reduce the spread of malaria.

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UNDERSTANDING MALARIA PREVENTION AMONG SEASONAL MIGRANT WORKERS FROM HIGHLAND ETHIOPIA

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To address the information gap in understanding malaria prevention among seasonally migrating workers to malaria endemic lowlands of Ethiopia during main crop harvesting season that coincides with major malaria transmission season, we have conducted knowledge, attitudes, and practices (KAP) study in two sugarcane plantations in the rift valley, central-east Ethiopia. This survey provides essential background information to understand situation of malaria in non-immune migrant workers and possible preventive strategies. Standardized questionnaires were distributed to seasonal workers from highland Ethiopia presenting for employment at recruitment centers at the Metehara and Wonji sugarcane plantations. Standard interview questionnaires were completed by 876 new recruits; 445 (50.8%) were returnees to the plantation for second or more seasons. Three hundred eighty seven (44.2%) of the interviewee had one or more attack of historical malaria in their life time. Of those with history of malaria 335 (86.6%) were returning migrant workers who had the attack during their previous stay or within 4 weeks after leaving these plantations. Among all migrant workers 546 (62.3%) had information about risk of acquiring malaria at these plantations. Only 112 (12.8%) migrant workers knew one or more of malaria prevention measures although only 34 of them (3.9% of all migrant workers) sought pre-travel professional medical advice. Sixty six (7.5%) of all study subjects had knowledge about personal protection measures against mosquito bites, but only 7 (0.8%) carried mosquito repellents or insecticides. Only 15 (1.7%) of them had already taken prophylactic drugs up on arrival at the plantation. In conclusion, risk of malarial attack is extremely high for migrant workers. The KAP of migrant workers at malaria endemic plantation about malaria is alarmingly low. To reduce the rate of malaria attack to non-immune migrants and avoid morbidity and mortality from severe form of malaria, targeted health education and preventive interventions should be routine provisions to non-immune migrant seasonal workers upon arrival at malarious plantations.

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METABOLIC PROFILES AND MALARIA TRANSMISSION

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Plasmodium vivax malaria is a serious public health concern in the Amazonian city of Iquitos, Peru. One malaria control strategy that goes beyond vector control is blocking malaria transmission by trying to stop infection at a critical stage in the parasites life cycle. Since parasite infectivity, competence of the vector, and host factors all play a role in malaria transmission, understanding each of these mechanisms will help in the development of anti-malarial vaccines. This study looks at one host factor: the nutritional environment for the parasite inside the human host. The nutritional environment is the composition of macromolecules and nutrients in the human host and this study examines how variations of the nutritional environment affect malaria transmission. Because the parasite is dependent on the host for nutrition, the nutritional status of the host may affect parasite biology. For example, differences in diets influence lipid serum levels, and the malaria parasite must scavenge lipids entirely from its host to survive. Since parasite biology is most often studied *in vitro,* cultured parasites often cannot account for the slight biological variations of the human host, such as nutritional environment. Previous results show that the *P. falciparum* parasite exists in the human host in at least three distinct physiological states, apparently related to glycolytic growth, a starvation response and a general stress response. These metabolic states were due to the different nutritional environments in which the parasites lived. Therefore, the metabolic state of the malaria parasite *in vivo* likely plays an essential role in parasite transmission. Examining an existing cohort of 100 persons living in Iquitos with previous *P. vivax* malaria episodes, we used a questionnaire and food log to assess type of food eaten, amount of calories consumed, and amount of oil used. We also measured physical characteristics such as height, weight, and Body Mass Index. We will use this dietary data to study how trends in diet relate to gametocyte density and gametocyte infectivity for mosquitoes. Studying *ex vivo* parasite biology can give insight about disease outcome and transmissibility, host-pathogen interactions, and potential discoveries of new therapeutic targets.

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UTILIZATION OF MOBILE PHONES FOR SAFETY REPORTING ASSOCIATED WITH SYSTEMATIC SCREENING AND TREATMENT OF *PLASMODIUM FALCIPARUM* ASYMPTOMATIC CARRIERS WITH ARTEMETHER-LUMEFANTRINE IN A COMMUNITY SETTING IN AFRICA

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Malaria pharmacovigilance in developing countries is essential, but it is challenging. The use of mobile phones for safety monitoring during a two-arm, community-based study for Coartem (artemether-lumefantrine [AL]) administered for asymptomatic (following systematic screening by rapid diagnostic test [RDT]) or symptomatic *falciparum* malaria in a rural district

of Burkina Faso is described. Pharmacovigilance procedures required close collaboration among study personnel, local health care facility (LHF) team, and district hospital staff involved with the study. Mobile teams were periodically deployed to screen half of the population (intervention arm) by RDT. Asymptomatic carriers were treated with AL or alternative medication, with a follow-up at Day 7. Subsequently every fever case was to be assessed by RDT at the LHF for diagnosis of a symptomatic malaria episode, and if positive treated similarly with a follow-up at Day 7. Severe cases were transferred to the district hospital. Every serious adverse event (SAE) was notified to the Principal Investigator team by the LHF staff (usually a head nurse) using a mobile phone. Subsequently a study physician was dispatched to the LHF to collect data and transcribe them onto a SAE form in English within the required timeframe. Original training on GCP and safety reporting performed at the onset of the study emphasized the requirement to report SAEs in all subjects throughout the study duration, as well as AEs within 7 days following treatment with AL. Despite that, the quality of SAE reporting was inconsistent, diagnosis of severe malaria was suboptimal, and no pregnancies were reported over 3 months. In order to improve reporting, 5 training sessions tailored to different study personnel, as well as LHF and district hospital staff, were conducted in March 2011. Channels for detection of SAEs were identified and implemented. Retraining proved to be necessary to increase awareness of study procedures at the district hospital, improve fever case management at the LHF, and ensure pregnancy reporting.

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MALARIA CONTROL IN PAST AND IMPACT OF GLOBAL FUND ON REDUCING RATES IN BANGLADESH

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Following the Malaria Eradication Programme (MEP) in early 1960s, malaria incidence dropped from 10.8 per 100,000 in 1968 to 4.22 per 100,000 people in 1971. The MEP relied upon indoor residual spraying using DDT, active surveillance for new cases, and effective drug treatment. After independence of the country in 1971, the MEP declined with a rise of malaria to 60.44 per 100,000 people in 1976. The MEP, reconverted to the Malaria Control Programme (MCP) in 1977 focused on vector control in limited susceptible areas without active surveillance. The malaria trend remained static until the early 1990s when malaria cases further increased following the official ban and cessation of DDT-use. The Bangladesh Govt. and BRAC recently received global fund money near US$ 80 million in 2006 and 2009. The goal of the global fund proposals were to reduce malaria specific morbidity and mortality by 50% with provision of early quality diagnosis, effective treatment and to expand use of LLIN and IRS to achieve 100% coverage in three Chittagong Hill tracts that reports >80% of the malaria cases and >90% of the deaths each year. The present control efforts rely on passive case-detection, effective treatment, and provision of indoor insecticide-treated nets. The global fund project has resulted in reducing reported mortality from 501 in 2005 to 37 in 2010; malaria incidence initially escalated from 48,121 in 2005 to 84,690 in 2008-due to improved detection at the community level but has started dropping consistently in the past 2 years 63,873 in 2009 and 55,873 cases in 2010. It is not clear if the decline in number of malaria cases is solely result of the Global Fund activities, the result of climate variability, or is even real. In addition to the passive reporting of the government our early findings from active malaria surveillance site amongst a population of 20,000 indicate that over 60% of the cases infections were asymptomatic. Strategies that identify and treat this large asymptomatic malaria positive population may be necessary to reduce transmission and sustain gains in malaria reduction.

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SYSTEMATIC SCREENING AND TREATMENT WITH ARTEMETHER-LUMEFANTRINE OF *PLASMODIUM FALCIPARUM* ASYMPTOMATIC CARRIERS IN A COMMUNITY SETTING IN AFRICA: IMPLEMENTATION PLAN

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Despite nationwide adoption of artemisinin-based combination therapy, and associated decline in malaria-related deaths, complementary interventions are still required to further reduce the disease burden. This 18-cluster (9 intervention clusters in villages; 9 control), randomized, single-center, controlled, parallel, prospective study will evaluate the impact of systematic treatment of asymptomatic carriers (ACs) of asexual forms of *Plasmodium falciparum* with artemether 20 mg-lumefantrine 120 mg (AL, Coartem/Coartem Dispersible, BID for 3 consecutive days) in approximately 9000-14000 subjects (male/female adults, children, and infants) from a community setting in Africa. The primary objectives are to evaluate whether treatment of *P. falciparum* ACs is associated with a lower number of symptomatic malaria episodes, RDT confirmed per person-year over a 12-month follow-up period and an improvement in hemoglobin levels after 28 days. Subjects will be excluded from receiving AL if they have severe malaria, known disturbances of electrolyte balance, history of congenital QTc prolongation or sudden death, body weight <5 kg, hypersensitivity to AL, or if they are in the first trimester of pregnancy. Those subjects will be treated with alternative drugs per current national guidelines. Responsibilities of the investigator's central site include microscopy, data entry, source data archiving, and supervision of the Demographic Surveillance System (DSS). DSS will monitor each cluster population every 2 months during the study for births, deaths, and in/out migrations; and provide an up-to-date demographic status of the study population. A mobile team supervised by the principal investigator will be supported by community healthcare workers (CHWs), a lead CHW, and a local healthcare facility for different study procedures. A unique permanent identification number will be assigned to each inhabitant. If the reduction of ACs and disease burden is confirmed, policymakers may consider this approach in the surveillance strategies being implemented by malaria control programs across Africa.

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NATURAL ENVIRONMENTAL AND HUMAN SOCIAL FACTORS DETERMINE PATTERNS OF CHILDHOOD MALARIA RISK IN MALAWI

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Plasmodium infection and malaria disease result from a combination of physiological, behavioral and environmental factors in the context of human adaptation. We studied patterns of infection in Malawian children to characterize disease patterns and devise predictive risk models using health and GIS datasets. A household-level, geolocated malaria indicator survey of ~7,200 households was performed during 2007. Locations of Malawi MOH-supported health facilities were obtained. Other GIS layers for roads, waterways, elevation and landcover were obtained from DIVA-GIS (www.diva-gis.com). Distances from residences to nearest health facilities, roads and water were calculated, and bivariate associations of all covariates and malaria-positive households were evaluated. From the survey data, we selected an optimal model through Akaike's Information Criterion (AIC) to predict probability of *Plasmodium* infection for all locations. Comparisons suggested that infected vs. non-infected children resided further away from health services (7.55 vs 6.05 km) and roads

(1.25 vs 0.9 km), at lower elevations (717 vs. 888 m) and nearer to water bodies (1.09 vs 1.26 km). An optimal logistic regression model included distance to health facilities (OR 8.73 [4.96,15.35]), roads (OR 4.48 [2.19,9.16]), water (OR 0.27 [0.15,0.50]), elevation (OR:0.998 [0.997,0.999]) and landcover categories, including cultivated land (ref), forest (OR: 1.04 [1.01,1.07]), and artificial surfaces (0.96 [0.92,1.00]). Our model indicates that malaria infections are best predicted by a combination of vector-based and human factors. Conditions favorable to vector reproduction increased infection risk, but health facilities and infrastructure modified this relationship, likely through a combination of prompt treatment, vector management and preventative interventions (e.g. ITNs). The resulting comprehensive map of malaria risk in Malawi showed highest risk among households along Lake Malawi, the Lower Shire basin and those proximal to game parks. Further efforts to create predictive models of malaria risk should not ignore important human-vector-environmental interactions.

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DIFFERENTIAL PROTEOMIC STUDY OF *PLASMODIUM FALCIPARUM*-INFECTED AND NON-INFECTED SALIVARY GLANDS OF *ANOPHELES GAMBIAE*: WHAT CONSEQUENCES FOR THE MALARIA TRANSMISSION?

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Malaria is a disease caused by parasites *Plasmodium* genus transmit by *Anopheles* mosquitoes during the blood feeding. During this blood meal, saliva and parasites are injected in the vertebrate host skin. Salivary molecules possess a pharmacologic role and proprieties of immunomodulation allowing a correct blood feeding and to fight the acquired immune response of host to bites. This saliva is also involved in the human-vector relationships. When the salivary glands are infected, the parasite modifies the vector biology. In the literature it has been demonstrated that the expression of salivary proteins of *Anopheles gambiae* infected by *Plasmodium berghei* are modified. Here we investigate what modifications in salivary glands of *An. gambiae* are occurred during the infection by *P. falciparum*. To assess this question we are comparing the salivary extracts of *An. gambiae* infected and not by proteomic approach. Experimental infections of *An. gambiae* by *P. falciparum* are carried out in Cameroon and salivary glands are dissected 14 days post-infection. The quantitative-PCR allowed to quantify the *P. falciparum* infection into the salivary glands. Then two-dimensional electrophoresis are underway with different pools of non-infected versus infected salivary glands. The differential analysis could show that several components are over- or down-expressed during the infection. Mass spectrometry analysis could know what proteins have a modified expression. These results could show that the parasite induces surely modifications of salivary proteins, certainly favoring the transmission. This study allows to have more knowledge about the relationships between the vector and his host and to develop new tools to evaluate the risk of malaria like a immuno-epidemiologic biomarker. These observations represent important new applications for the vector control strategies.

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TERRAIN DIFFERENCES AFFECT MALARIA VECTOR DISTRIBUTION IN WESTERN KENYA HIGHLANDS

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One of the key drivers to malaria in African highlands is the terrain characteristics because topography affects climate, hydrological characteristics and thus larval habitat availability and stability and micro-climate of the highland. This study examined the impact of three different terrains (U-shaped, V-shaped valleys and plateaus) on malaria transmission. A model based on the terrains of each terrain and the precipitation thresholds was established. Two parameters that define the terrains are the size of the area at the bottom of the valley with a zero or close to zero side slope and slope of the river flow direction. These numerical values were determined by geospatial analysis with GIS techniques as the major parameter that affects drainage, habitat stability, vector productivity and rate of malaria transmission. The primary results indicate that the U-shape valley has 3-fold more *Anopheles gambiae* female mosquitoes, the principal malaria vector in western Kenyan highlands, than the V-shape valley system. These support our hypothesis that the greater stability and productivity of the breeding habitats in the U-shape valleys compared to the V-shape valley.

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THE TANZANIAN NATIONAL VOUCHER SCHEME: IMPROVING TAKE-UP BY REDUCING THE TOP-UP PRICE PAID BY VOUCHER BENEFICIARIES

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Since 2004, the Tanzanian National Voucher Scheme (TNVS) has made insecticide treated nets widely available and accessible to pregnant women and infants through a donor supported voucher system that subsidizes the cost of nets purchased in commercial retail outlets. Between 2005 and 2008 voucher nets became less affordable as the top-up amount paid by beneficiaries rose in-line with increasing net prices, reaching the equivalent of nearly 2 USD by 2008. As a result, the proportion of vouchers redeemed for a net fell from 88% in 2005 to 54% in early 2009. 20% of voucher recipients in 2008 cited lack of money as the reason for not using the voucher and significant inequity in redemption existed. In late 2009, this inequity was addressed by increasing the value of the voucher and fixing the cash top-up amount to ~ 0.35 USD. Quarterly voucher redemption rates were tracked from July 2004 to March 2011. Redemption rates were calculated by dividing the number of redeemed vouchers with matching returned stubs by the total number of voucher stubs returned in a given time period. Between the January 2010 and March 2011, Pregnant Women and Infant Voucher redemption rates returned to their initial levels by rising from 54% to 82% and 51% to 84%, respectively. Compared to the 15-month period prior to implementation of the voucher upgrade, the overall number of redeemed vouchers rose by 29% from 1.3 to 1.7 million. The number of vouchers redeemed in rural areas increased by 46% and for urban areas the increase was 28%. These improvements occurred during a period when more than 20 million free long lasting insecticidal nets were issued in two mass campaigns, allaying earlier concerns that free net distribution would weaken the TNVS, which relies on a small co-payment. Thus, the current

TNVS approach may contribute towards a keep-up mechanism to maintain high net ownership among targeted risk groups across socioeconomic groups after free net mass distributions are completed.

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POSITIVE DEVIANCE: AN INNOVATIVE APPROACH TO IMPROVE MALARIA PREVENTION AND TREATMENT PRACTICES AMONG MOBILE AND MIGRANT WORKERS IN CAMBODIA

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Reaching mobile and migrant populations is one of the key strategies in the containment and elimination of artemisinin resistance in the Greater Mekong Subregion (GMS). Positive Deviance (PD) is an asset-based behaviour change approach with the underlying notion that every community has certain individuals (*positive deviants* or champions) whose malaria prevention and treatment practices result in better health outcomes than their neighbours. Malaria Consortium (MC) supported Cambodia's National Malaria Programme to pilot PD among residents and migrants in three villages in Sampov Loun district. The PD pilot aims to identify and promote good health seeking practices in both communities. The baseline survey conducted in Aug 2010 (n=309), suggested that knowledge about malaria and prevention were high in both communities but health-seeking behaviour for fever could be improved (residents 44.4%; migrant 33.3%), The PD process included 6 steps: pre-orientation meeting, community orientation, situation analysis, PD inquiry, participatory analysis, and community feedback. During the process, 13 in-depth interviews and 6 group discussions were conducted to identify the champions. For example, we identified a female migrant worker who never gets malaria by always sleeping under a bed net, wearing long sleeved clothes, covering her legs with a scarf while watching TV, and immediately going to the health centre when ill. All PD practices were shared with other community members through a 6 month PD-informed intervention which included training of volunteers, interactive health education sessions, role plays, art competitions and an advocacy seminar. A one-year follow up survey will be conducted in Aug 2011 to better evaluate this intervention, but preliminary results suggest that PD can serve as 1) a malaria intervention targeting migrants; 2) an alternative or supplementary method to deliver existing BCC/IEC interventions; and 3) an innovative model to promote community-based, bottom-up approaches.

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IDENTIFICATION OF NOVEL BINDING PARTNERS OF ANTI-*PLASMODIUM* IMMUNE FACTORS

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In *Anopheles gambiae* mosquitoes, defense against *Plasmodium* parasites is controlled by the innate immune system. We have previously identified a number of *Plasmodium* effector molecules through the use of microarray analysis and RNAi mediated gene silencing assays. However, their exact mechanism of action remains largely unknown. As a first step to further elucidate the signaling cascades and protein complexes involved in anti-*Plasmodium* defenses, we utilized the yeast two hybrid system to identify novel binding partners of the selected anti-*Plasmodium* effectors AGMDL1, APL1A, APL1C, FBN9, FBN39 and LLRD7. Thus far, we have discovered a number of different interacting proteins some of whose functions in immunity have not been previously described. RNAi studies showed that many of these *Plasmodium* effector interacting proteins also play a role in controlling *Plasmodium* development in the mosquito midgut. We are currently using biochemical methods to further characterize and investigate the role of these newly discovered protein-protein interactions in the innate immune response against *Plasmodium* infection.

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TRANSCRIPTOMIC AND FUNCTIONAL ANALYSIS OF DENGUE VIRUS INFECTION IN THE *AEDES AEGYPTI* SALIVARY GLAND

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The *Aedes aegypti* salivary gland plays a crucial role in dengue virus (DENV) transmission. Infection of the salivary gland is essential for horizontal DENV transmission to occur, and the gland also produces numerous immune-modulatory, vasodilatory, and anti-coagulant molecules that facilitate bloodmeal acquisition. To characterize the anti-DENV immune response in this organ, we performed a genome-wide microarray analysis of the naïve and DENV-responsive *A. aegypti* salivary gland transcriptomes. Two candidate genes identified from this analysis had the ability to modulate mosquito midgut DENV titers: RNAi-mediated knockdown of MD6 (an MD2-like gene family member) significantly decreased DENV titers, while knockdown of LAP4 (a gene encoding a leucine-rich repeat-containing protein) significantly increased DENV titers. Similar assays are currently being performed to evaluate their effect on salivary gland DENV replication. Our microarray analysis also identified two salivary gland-enriched odorant-binding proteins (OBPs) that were also induced by DENV infection in this organ. Knockdown of these OBPs in the salivary gland decreased the percentage of mosquitoes that probed on an anesthetized mouse. In addition, mosquitoes that did probe took longer to initiate the probe, and spent more time probing before successfully acquiring a bloodmeal. Thus, DENV infection in the salivary gland not only regulates genes that modulate virus replication, but also genes that potentially affect bloodmeal acquisition (and hence DENV transmission) by modifying mosquito host-seeking or probing behavior. Further characterization of these genes will yield a clearer picture of host-pathogen interactions in this poorly-studied organ.

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THE SIGNIFICANCE OF A MOSQUITO HYPER-VARIABLE PATTERN RECOGNITION RECEPTOR, *AGDSCAM*, IN THE ANTI-*PLASMODIUM* DEFENSE

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The innate immune system of the mosquito, unlike that of vertebrates, appears to lack the adaptive immunity and immunological memory, which relies on the limited numbers of germ line-encoded pattern recognition receptors to generate the specificity towards the pathogen recognition. The studies of the molecular mechanisms that determine the recognition of the pathogens are of the biggest interest. AgDscam, *Anopheles gambiae* down syndrome cell adhesion molecule, which have the potential to generate 31,920 alternative splice forms with different interaction specificities. We have shown that AgDscam is an essential hypervariable receptor of the *Anopheles gambiae* immune surveillance system, which produces splice form repertoires that are pathogen challenge-specific. In this study, we have used siRNA gene silencing approach to target the specific isoforms of AgDscam and are able to show that AgDscam's anti-*Plasmodium* responses are splice-form specific. We furthermore show this defense specificity by using transgenic *A. stephensi* mosquitoes which are overexpressing either *Plasmodium falciparum* or *P. berghei* specific spliceforms upon a blood meal. Similarly, transgenic mosquitoes also showed specificities in controlling microbial proliferation in their midguts.

At cellular level, through confocal microscopy we show co-localization of AgDscam with both *P. falciparum* and *P. berghei* which suggests that AgDscam is directly associated with the parasites. Transgenic mosquitoes with overexpression of *P. falciparum* specific isoform has more abundant protein co-localized with the *P. falciparum*. Gene expression analyses suggests that the Toll and Imd immune pathways are involved in the regulation of alternative splicing of AgDscam. Preliminary experiments show that several putative immune responsive putative splicing factors are involved in the regulation of AgDscam splicing. Further detailed studies are undergoing to investigate the pathogen binding specificities of different spliceform through an *in vitro* recombinant protein strategy.

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APPLICATION OF TANDEM MASS SPECTROMETRY FOR ARGININE QUANTIFICATION IN *AEDES AEGYPTI* FEMALES, THE MAIN VECTORS OF DENGUE AND YELLOW FEVER

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In order to gain insights into uricolysis and argininolysis, two metabolic pathways involved in the urea synthesis in *Aedes aegypti*, it is necessary to develop an efficient method to identify and quantify arginine (Arg) in mosquitoes. In our laboratory, a procedure used for the identification of Arg in urea disorders in newborn babies was adapted to identifying and quantifying Arg in mosquito excreta by tandem mass spectrometry. We derivatized 14N-Arg and 15N2-Arg (labeled guanidine) as isobutyl esters and then the fragmentation patterns of both compounds were analyzed by electrospray ionization tandem mass spectrometry. When isobutyl esters of 14N-Arg (m/z=231) or labeled Arg (m/z=233) are fragmented, neutral losses of 161 Da, or 163 Da respectively, occur and produce fragments of m/z=70. Based on these studies, the mosquito excreta from blood fed females were collected and mixed with 15N2-Arg (an internal standard). The samples were then derivatized as isobutyl esters and sprayed into a Q-trap 4000 mass spectrometer. The levels of Arg in the excreta of a single mosquito, at different times after a blood meal, can be successfully monitored at 30 eV by multiple reaction monitoring scans. This method provides an efficient and rapid tool to quantify Arg in mosquitoes, as well as in other insects whose small size severely limits the use of more conventional biochemical methods of analysis.

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OVARY ECDYSTEROIDOGENIC HORMONE (OEH) STIMULATES BLOOD DIGESTION AND EGG MATURATION IN FEMALE *AEDES AEGYPTI* INDEPENDENT OF INSULIN SIGNALING

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The yellow fever mosquito *Aedes aegypti* is an important vector of human disease pathogens and is a model for invertebrate endocrinology. Blood ingestion by females stimulates the release of two types of neuropeptides, Ovary Ecdysteroidogenic Hormone (OEH) and an insulin-like peptide (ILP3), which activate the ovaries to produce ecdysteroid hormones. We recently reported that ILP3 directly binds to the mosquito insulin receptor (IR) and regulates vitellogenesis and blood digestion. Its activation of these processes is greatly amplified by amino acid sensing and activation of the target of rapamycin (TOR) pathway. We now show that OEH similarly regulates these processes and depends on amplification by amino acids and TOR signaling. However, OEH does not activate IR phosphorylation, and its activity is not affected by a specific IR inhibitor. Together these results indicate that OEH and ILPs both regulate egg maturation in this species, but their activity involves interactions with different receptors.

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TRANSLATION REGULATION IN RESPONSE TO *PLASMODIUM FALCIPARUM* INFECTION IN *ANOPHELES GAMBIAE*

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Midgut invasion is the greatest bottleneck among the mosquito host stages of the *Plasmodium* lifecycle as a rapidly responding immune system labels ookinetes and recruits killing factors from the midgut and surrounding tissues, dramatically reducing the population of invading ookinetes before they can successfully traverse the midgut epithelium. As it is so crucial to *Plasmodium* survival, the midgut bottleneck represents one of the best points in the *Plasmodium* lifecycle to combat malaria. A better understanding of genetic regulation of the vector in response to parasitization at this point may provide critical targets and enhance strategies for impacting parasites at one of their weakest links, yet little is known at the level of translation. We isolated *An. gambiae* midguts at 24 hours after a bloodmeal with *P. falciparum* gametocytes, followed by sucrose gradient fractionation to separate transcripts based upon association with polysomes. Transcriptome sequencing has provided over 32 million reads representing over 10,000 different transcripts. We have found 577 genes where transcriptional change is insignificant (2fold) between infected and uninfected samples, with a $p<0.05$. This led to the identification of 11 different transcripts involved in immune response that underwent significantly higher polysomal association and therefore more active translation during infection. REL-2, a NF-kB-like transcription factor, was more actively translated in response to infection while insignificant change occurred at the steady state mRNA level, which supports previous findings that REL-2 plays important roles in the immune response to *Plasmodium*. We are currently examining the extent to which the different isoforms of REL-2 are involved. IKK2, an Anopheline ortholog of a Drosophila Imd-pathway component linked to REL-2, was also upregulated at the translational level. Our findings support the hypothesis that the antimalarial response of *Anopheles* occurs at both the transcriptional and translational level.

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HUMAN DENGUE-RESISTANT GENE, FKBP1 IS FUNCTIONALLY CONSERVED IN *AEDES AEGYPTI*, YELLOW FEVER MOSQUITO

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There are 22 human genes that encode resistant factors to both West Nile virus (WNV) and dengue virus (DV). These genes were identified by genome-wide RNAi screening using HeLa cells, in which RNAi silencing of those 22 individual genes caused DV titers to increase. We investigated orthology and functional conservation of the 22 human dengue virus resistant (DVR) genes in the yellow fever mosquito, *Aedes aegypti*. Homology searches of the 22 human DVR genes identified 12 orthologs in *Ae. aegypti*. Functional conservation of the 12 *Ae. aegypti* DVR orthologs was examined by siRNA silencing and co-infection of dengue virus in an *Ae. aegypti* cell line (Aag2). After Aag2 cells were transfected with siRNAs individually targeting each of 12 DVR orthologs and subsequently infected with DV (NGC Type 2, m.o.i.= 0.05), the cell culture media were harvested to determine the DV titers by a standard plaque assay at 3 days post infection. Among the 12 DVR orthologs tested, the knockdown of only one candidate, fk506-binding protein 1 (FKBP1), promoted dengue replication by 24 and 51% compared to the control group in two independent experiments, each with six replicates (*t* test, $P<0.01$). This result suggests that Aag2 cells knocked down for FKBP1 became more susceptible to dengue virus infection than the control. Apparently, the human DVR gene, FKBP1B, is functionally conserved in *Ae. aegypti*. Humans and mosquitoes may share a common mechanism(s) of dengue resistance mediated via FKBP. FKBP1s are immunophilins that bind to

immune suppressive drugs such as FK506 or rapamycin to suppress T-cell activation and proliferation via deactivation of NF-AT or NF-κB in mammals. Both human and *Ae. aegypti* FKBP1 proteins consist of 108 amino acids and share 67% amino acid identity between them. High sequence homology and functional conservation of FKBP1 as a DVR in humans and *Ae. aegypti* should warrant further studies to elucidate the inhibitory mechanism of FKBP1 against DV in humans and mosquitoes. These studies may provide a novel paradigm to control dengue transmission.

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JUVENILE HORMONE III SUPPRESSES FOXO IN THE FAT BODY AND REDUCES FAT ACCUMULATION IN THE DIAPAUSING MOSQUITO, *CULEX PIPIENS*

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Juvenile hormone III (JH) controls a variety of physiological and developmental events including diapause and nutrient metabolism. The focal point of endocrine regulation in adult reproductive diapause is initiated by a halt of JH synthesis. The other key molecular event is the signaling pathway from insulin to forkhead transcription factor (FOXO) in diapause females. We hypothesized that halt of JH synthesis is related to activation of FOXO, which results in increasing lipid reserves in the fat body at the onset of the diapause program. In this study, the full length sequence of the foxo gene was characterized, and the protein abundance pattern of the foxo gene product was analyzed by immunoblotting and immunohistochemistry. FOXO was predominantly present in the fat body of diapausing females and to a less extent in the fat body of nondiapausing females; it was either absent or very weakly present in other tissues one week after adult eclosion. Interestingly, when we topically applied JH to diapause-destined females after adult eclosion, FOXO was suppressed and fat accumulation was reduced. In contrast, the activity of nucleoli, observed by confocal microscopy with DAPI-staining, was higher in fat body cells of diapausing females that received a topical application of JH, as compared to those not receiving JH.

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BROAD SCREENING OF *ANOPHELES*` MICROBIOTA WITH ANTI-*PLASMODIUM* EFFECT

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Malaria is disease caused by parasites of the *Plasmodium* genus that are transmitted by anopheline mosquitoes. For successful malaria transmission the parasite needs to complete a complex life cycle in their vectors. *Plasmodium* spends its firsts 24 hours in the mosquito midgut lumen. During this period the parasites pass through different developmental stages and are exposed to the mosquito's immune responses, digestive enzymes and the resident microbial flora before they can invade the midgut epithelial cells. We have identified a variety of bacterial strains from wild caught mosquito populations and showed that an Enterobacter species has a potent anti-*Plasmodium* activity. Here we present a comprehensive analysis of anti-*Plasmodium* activity of seven different field isolates of bacteria: *Comamonas,* two *Pseudomonas, Acinetobacter, Serratia, Chryseobacterium* and *Pantoea*. *In vitro* and *in vivo* experiments showed that different strains of bacteria are capable of inhibiting *Plasmodium* development at different stages and through different mechanisms. Some of these bacteria species may be used for the development of powerful biocontrol strategies to block malaria transmission.

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THE EFFECT OF CHOLESTEROL ON *WOLBACHIA*-MEDIATED VIRAL INTERFERENCE AND HOST FECUNDITY IN THE DENGUE MOSQUITO *AEDES AEGYPTI*

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Infection of the dengue mosquito *Aedes aegypti* with the bacterial endosymbiont *Wolbachia pipientis* induces a number of physiological manipulations in the host. These include interference against arboviruses, including dengue virus (DENV) and also reduced fecundity and egg viability after bloodfeeding on non-human hosts. These manipulations are potentially the product of competition with the host for scarce nutritional resources. One such resource is cholesterol, which is only available to adult mosquitoes after a bloodmeal. Cholesterol is critical to cellular signalling and membrane organisation, to egg and larval development in mosquitoes, vacuole formation in endosymbiotic bacteria, and viral entry and replication in certain arboviruses. To observe if cholesterol was involved in viral interference and fecundity in *Wolbachia*-infected *A. aegypti* we fed groups of female LEWIS rats on four different cholesterol-enriched diets for 10 weeks, and then obtained their blood via cardiac puncture. Blood cholesterol levels of rat and human (control) blood were estimated using the Amplex Red Cholesterol Quantification Kit (Molecular Probes, Invitrogen). These bloods were then fed to *Wolb*+ and *Wolb*- mosquitoes and fecundity and egg viability were observed over four replicate experiments. Results indicated that cholesterol had no significant, repeatable effect on either fecundity or egg viability, suggesting it is not involved in the reduced fecundity manipulation. To examine the effect on viral interference, aliquots of rat blood were dosed with DENV and fed to *Wolb*+ and *Wolb*- mosquitoes over three replicate experiments. At 14 days post-feeding, DENV copy numbers were quantified using qPCR. Results indicated that for *Wolb*- mosquitoes, cholesterol levels had no effect on DENV load. The majority of *Wolb*+ mosquitoes were refractory to DENV regardless of cholesterol levels, however a small subset showed DENV breakthrough with a strong negative correlation between cholesterol levels and viral load suggesting a possible role for cholesterol in *Wolbachia*-mediated viral interference.

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THE CHARACTERIZATION OF GLUTAMATE-GATED CHLORIDE CHANNELS FROM *ANOPHELES GAMBIAE* AS A POTENTIAL INSECTICIDAL TARGET

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Malaria is one of the most devastating mosquito-borne diseases, causing millions of deaths annually. Current malaria parasite transmission control methods are heavily reliant on insecticide treatment of walls and bednets. This has fostered insecticide resistance, and focuses control primarily against endophagic mosquitoes. There is a need to develop insecticides with new modes of actions and the ability to control the spread of malaria parasites from exophagic mosquitoes. We have recently demonstrated the potential of mass drug administrations of the anthelmintic drug ivermectin to control malaria parasite transmission. Glutamate-gated chloride channels (GluCls) are the target of ivermectin, and other macrocyclic lactones. These drugs agonize GluCls located on muscle tissue causing paralysis and death. The purpose of this study is to assess GluCls as a target of mosquitocidal drugs and vaccines. We have cloned GluCl from *Anopheles gambiae*, the primary vector of malaria in sub-Saharan Africa. To characterize channel activity we have expressed the channel in the *Xenopus laevis* oocyte. Using whole-cell electrophysiological analysis we obtained initial evidence of channel function in response to glutamate and

ivermectin. To more precisely measure channel activity we have expressed the channel in the C6/36 mosquito cell line. Using outside-out voltage clamp technique and the piezo liquid switch perfusion system we have obtained initial evidence of channel activity in response to glutamate. To test AgGluCl as a potential target for a mosquitocidal vaccine, we fed *A. gambiae* blood meals spiked with increasing titers of a polyclonal antibody against the extracellular N-terminal domain of GluCl. Anti-GluCl antibodies caused rapid paralysis and death of nearly all of mosquitoes that blood fed on the highest concentration (3 mg/ml), and more than 50% of the mosquitoes that blood fed on the 1.5 mg/ml antibody concentration. In conclusion, we have initial *in vitro* and *in vivo* evidence that *An. gambiae* GluCls are a potential target for mosquitocidal drugs and vaccines.

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THE EFFECT OF EXPRESSING APOPTOSIS-REGULATING GENES ON SINDBIS VIRUS REPLICATION IN THE MOSQUITO VECTOR *AEDES AEGYPTI*

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Apoptosis is known to be a defense against some viruses in insects and mammals. The role of apoptosis in mosquito immunity against arboviruses is largely unexplored; although some studies have suggested a correlation between apoptosis and resistance to infection, no direct evidence exists supporting a causal relationship. The mosquito *Aedes aegypti* is an important vector for yellow fever and dengue. Because of its ability to be engineered to express foreign genes, Sindbis virus (SINV; Togaviridae) was used to study the possible role of apoptosis in *A. aegypti* immunity against arboviruses. A series of infectious SINV clones based on the construct p5'dsMRE16ic was engineered to express pro-apoptotic or anti-apoptotic genes from a duplicated viral subgenomic promoter. Control virus clones were also constructed containing the same inserts but in antisense orientation. A previous study demonstrated that the clones expressing apoptosis-regulating genes either induced or inhibited apoptosis as expected in cultured *A. albopictus* cells. In this study, adult female *A. aegypti* were infected by artificial blood meal containing the recombinant SINV clones, and virus infection was analyzed at various times post-infection in midguts by immunofluorescence (IFA) against the viral E2 protein. Viral replication was also monitored by titering the amount of infectious virus in individual mosquitoes. Virus clones expressing pro-apoptotic factors caused increased caspase activity and TUNEL staining in midgut compared to controls, indicating that apoptosis was stimulated by these virus clones. IFA and viral titer results indicated that infection with SINV clones expressing pro-apoptotic genes decreased the rate and spread of virus infection in the mosquito compared to controls. The results suggest that if apoptosis occurs in infected cells, it may be able to play a role in defense against arbovirus infection in mosquitoes.

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XANTHURENIC ACID RECEPTOR STUDIES IN GAMETOCYTES OF *PLASMODIUM FALCIPARUM*

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Xanthurenic acid (XA) had been identified as the gametocyte activating factor (GAF), which may play a major role in *Plasmodium* gametogenesis in the gut of the mosquito. There is evidence that XA may stimulate this process via a G-protein mechanism. XA may bind to a G-protein receptor, and this complex in turn may stimulate a GTP dependent kinase activity begining a cascade of events for signal transduction. It is our hypothesis that XA ligand binds to a 'XA binding G-protein associated receptor'. *P. falciparum* strain 3D7, is known to be responsive to XA stimulation and was used to obtain the sexual forms (gametocytes). Gametocytes/RBC preparations were produced and tested in XA radio-ligand binding assay along with RBC controls. It was found that XA was binding significantly to the control RBCs and hence we started examining the XA binding to RBC-membrane free gametocytes. Initial experiments showed a large difference in binding of XA to the naked gametocytes *vs.* RBCs. Cold XA did abolish ^{3}H-XA binding at the concentration tested (100:1 cold to hot) indicating the binding was specific to a XA receptor. Saturation experiments with RBC membrane-free gametocytes showed that concentrations of XA at 5 µM did not achieve saturation with 0.2 x 10^{5} gametocytes. With 200,000 gametocytes at the highest concentration tested (5 µM) this would indicate either a very high concentration of receptor (6x10^{6}/gametocyte) or one with a low binding affinity. The binding of XA was temperature dependent indicating that some energy requiring step may be necessary to support the receptor binding. If identified the XA receptor can potentially be targeted for vaccine and drug development.

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SALIVARY GLAND SURFACE (SGS) PROTEINS FORM A MAJOR COMPONENT AND IMMUNOGEN OF MOSQUITO SALIVA

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The SGSs (salivary gland surface) are a family of large proteins, some of which are expressed in the salivary glands of female mosquitoes. Although little else is known about them, mosquito SGSs appear to have been horizontally transferred into the mosquito genome from *Wolbachia* proteobacteria and have been implicated in salivary gland invasion by *Plasmodium* parasites. Using proteomic and immunologic analyses, we show that the expression of *Anopheles gambiae* SGS4 and SGS5 is associated with blood feeding and that SGSs form a major component of the saliva of both *An. gambiae* and *Aedes aegypti*. Western blots and RT-PCR showed that *Anopheles* SGS4 and SGS5 expression is salivary gland and female specific, increases with age, increases after blood feeding, and SGS4 and SGS5 levels fluctuate in a circadian manner. Immunohistochemistry and Western blots showed that *An. gambiae* SGS4 and SGS5 localize primarily in the distal lateral lobes of the salivary glands, with lower levels also found in the secretory duct of the proximal region. Additional Western blot and bioinformatic analyses suggest that SGSs are secreted in a nonclassical pathway which includes proteolysis, yielding a ≈300 kDa secreted N-terminal fragment. SDS-PAGE, Western blots, "bite blots", and immunization via mosquito bites revealed that SGSs form a major protein component and immunogen in *Anopheles* saliva and that they are likely the most prevalent proteins (by mass) in *Ae. aegypti* saliva. SGS's incriminating mass, in combination with data from others, suggest that SGSs might play an immunomodulatory role during mosquito blood feeding.

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AEDES AEGYPTI IMMUNE SYSTEM ACTIVATION BY THE INTRACELLULAR BACTERIUM *WOLBACHIA PIPIENTIS* AND INTERFERENCE WITH RNA VIRUSES REPLICATION

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The intracellular bacterium *Wolbachia pipientis* naturally infects up to 70% of all insect species. Its success can be attributed to the ability of *Wolbachia* to manipulate the reproduction of its hosts to enhance its own transmission. This transmission advantage might also be enhanced by its ability to confer protection to insect hosts agains a range of pathogens. The mosquito *Aedes aegypti* is not naturally infected by *Wolbachia* but in recent years it has been artificially infected with a number of different *Wolbachia* strains which block transmission of both dengue and chikungunya viruses (*w*Mel and *w*MelPop). The molecular origin of "*Wolbachia*-mediated-protection" in its natural host *D. melanogaster* as well as in its heterologous host *A. aegypti* is unclear. Some studies suggest that *Wolbachia* primes the insect innate immune system and others

that the bacterium competes directly with viruses for limiting subcellular resources. To further understand this issue we undertook a comparative analysis of the *A. aegypti* transcriptome in response to *w*MelPop and *w*Mel infections. 210 gene transcripts were affected by both *Wolbachia* strains. Among them an elevated number of immune genes were activated. Because *w*MelPop and *w*Mel also protect their natural host *Drosophila melanogaster* against RNA viruses, a subset of immune gene transcripts were then analyzed in flies in response to the two *Wolbachia* strains. No consistent immune activation was detected. This comparison in both native and heterologous hosts suggests that immune priming by *Wolbachia* is an artefact due to its recent introduction into *A. aegypti*. This immune response might contribute to the pathogen protection effect, but our data indicate that the fundamental mechanism is more likely to be related to competition for limiting resources between *Wolbachia* and RNA viruses.

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QUANTITATIVE PROTEOMIC ANALYSIS OF O'NYONG-NYONG VIRUS INFECTION IN THE *ANOPHELES GAMBIAE* MIDGUT

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Multiplex quantitative proteomics has become an invaluable tool in assessing genome-wide protein modulations of organisms in response to pathogens. The protein expression patterns of mosquitoes stemming from arbovirus infection are particularly intriguing. These studies not only offer insight into host-virus interactions, but may also reveal pathogenesis factors significant to human disease; for example, differences in human and mosquito-vector responses to the same alphavirus, may illuminate how apoptosis is induced in the former, but a persistent, controlled infection in the latter. The current study investigated protein expression profiles of *Anopheles gambiae* midgut tissue resulting from an alphavirus infection (o'nyong nyong virus, ONNV, Togaviridae). Three pools of 50 midguts were harvested from uninfected (control) and infected mosquitoes at six-days post infection. Total protein extracts were then comparatively quantified using 6-plex tandem mass tagging (TMT). As a result, 22 peptides were found to have been significantly modulated, either positively or negatively, in the ONNV-infected midguts compared to the control (Bonferroni adjusted $P<0.1$). Intriguingly, among those peptides identified was an ortholog of an FKBP-type peptide that was overexpressed in the ONNV-infected mosquito midgut. More interestingly, FKBP has recently been identified as a human resistance factor to both dengue and West Nile viruses, suggesting possible functional conservation of antiviral activity mediated via this protein. Also of interest were peptides associated with pathways known to be targeted by alphavirus nonstructural peptides, and those involved in modifying ion transport, which in mammalian cell culture is an early indicator of cytopathic effect. These and other peptide responses will be discussed in the context of mosquito-pathogen interactions, as well as their implications for future studies.

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EFFECTS OF HUMAN IGF1 ON LIFESPAN, IMMUNE SIGNALING AND PARASITE SURVIVAL IN THE MALARIA VECTOR *ANOPHELES STEPHENSI*

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The highly conserved insulin/IGF-like signaling (IIS) pathway regulates metabolism, development, lifespan, and immunity across a wide range of organisms. Previous studies in our laboratory show that human insulin ingested in the blood meal activates mosquito IIS, resulting in attenuated lifespan and increased malaria parasite infection. Since human IGF1 (hIGF1) is present at higher concentrations in blood than insulin and is closely tied to lifespan and immune processes, we predicted that hIGF1 could affect lifespan and parasite infection in *Anopheles stephensi*. Preliminary results showed that hIGF1 in the blood meal induced activation of FOXO and p70S6K, proteins of the PI3K branch of the IIS, in the *An. stephensi* midgut. This activation was dose dependent, with low levels of hIGF1 inducing greater levels of IIS activation than higher levels. In addition, low concentrations of hIGF1 extended mosquito lifespan by 5 days or 11.8% relative to controls, while higher levels of hIGF1 did not differ from the control treatment. Effects of hIGF1 treatment on asexual parasite growth and infectivity were determined with a standardized *in vitro* assay and with *An. stephensi* infection, respectively. Our data showed that while hIGF1 did not alter growth of asexual *P. falciparum*, it did alter malaria parasite development in the mosquito, presumably via IIS activation. We have demonstrated that the IIS pathway is a promising target to genetically alter mosquito lifespan or to block malaria parasite transmission. However, a more complete understanding of the naturally occurring ligands in blood that activate IIS will be necessary to optimize strategies for transgenesis.

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LEVEL OF ANTIBODIES ANTI- *AEDES AEGYPTI* SALIVA AND CLINICAL PRESENTATION OF DENGUE FEVER IN NORTE DE SANTANDER COLOMBIA

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Dengue is endemic in Colombia and it is highly prevalent in Norte de Santander where its main vector is *Aedes aegypti*. We evaluate the association between the levels of anti-*Ae. aegypti* saliva IgG and IgM antibodies in people with dengue fever and healthy individuals. We found that the level of IgG antibodies where significantly higher in people with confirmed dengue fever than in people with febrile syndrome and/or healthy individuals, in contrast to the level of IgM antibodies in which we did not found any significant difference among the study groups. Additionally, we found differences in the salivary proteins recognized by the pool of serum from these three groups. This results suggest that antibodies against *Ae. aegypti* saliva are useful markers for both mosquito bite exposure and risk for clinical dengue fever.

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HABITAT SUITABILITY AND SPATIAL DISTRIBUTION OF FIVE *ANOPHELES* SPECIES IN AMAZONIAN BRAZIL

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Availability of suitable larval aquatic habitat may strongly influence the distribution and population dynamics of anopheline mosquito malaria vectors (Dipera: Culicidae. Mosquitoes breed in a wide variety of terrestrial water accumulations, but individual species prefer particular types of habitats, with many environmental variables correlated with the presence or development quality of *Anopheles* larvae. Data on presence or absence of five species, *An. oryzalimnetes*, *An. marajoara*, *An. janconnae*, *An. triannulatus* and *An. nuneztovari* were used for analysis of associations based on physiochemical factors of the water, canopy coverage, shade and available resources. 54 aquatic habitats containing mosquito larvae were characterized in Pará and Roraima states. *An. triannulatus* and *An. nuneztovari* had the greatest overall occurrence across the distribution; however *An. marajoara* had the greatest overall abundance. A principal

components analysis was conducted to examine multifactor environmental effects on larval density. The most important factors associated with larval abundances were water quality, available resources, and to a lesser extent canopy protection, temperature and water movement account for 73% of the variance among habitats. Four of the five species appear to be more specialized, suggesting possible adaptive niches. Negative correlations of abundances and environmental variables were found for: available resources for *An. oryzalimnetes*; temperature and water movement for *An. marajoara;* canopy protection and water movement for *An. nuneztovari*; and water quality and canopy coverage for *An. janconnae*. *An. janconnae* also showed a positive correlation of abundance with available resources and water movement. *An. triannulatus* had no significant correlations suggesting it is a habitat generalist. Increasingly warm and variable climate is likely to increase the range and abundance of many insect vectors. Comparison and characterization of larval habitats is critical for understanding the spatial and temporal distribution patterns of anopheline species and for implementation of control strategies.

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MARK- RELEASE-RECAPTURE STUDY TO MEASURE DISPERSAL OF *AEDES ALBOPICTUS* IN CHIANG MAI PROVINCE, NORTHERN THAILAND

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Aedes albopictus is widely distributed throughout Thailand and plays an important role in transmitting viral diseases i.e dengue fever and chikungunya. Adult control is targeted within 100 meters around a patient house. To ascertain whether this limit is appropriate we conducted a mark-release-recapture study to measure dispersal of *Ae. albopictus* in the rural area of Chiang Mai northern Thailand, where this mosquito species is found in abundance. Male and female mosquitoes reared from wild-collected immature stages were marked with fluorescent dust. A total of 3,000 female and 1,000 male were replicated releases during dry (March 2010) and wet season (July 2010). Recapture sites were set at 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275 and 300 meters respectively. Collections were made 6 days post-release. In each recapture site one pair of collectors collecting the mosquito every 10 minutes by using the sweeping hand net and aspirator. Collection started from 6.00 am to 6.00 pm. during 6 day post release. As a total of 177 and 36 female (5.9%, 1.2%), 36 and 11 male (3.6 %, 1.1 %) were recaptured in wet and dry season respectively. In wet season the furthest being caught was 275 meters from release point and the mean distance traveled was 153 m. In dry season, mosquitoes fly shorter of 150 meters furthest from the release point. There was a significant tendency for dispersal in wet season, and the recapture dates lasted till 6 days while it lasted 4 days post release in dry season. The result suggests that adulticiding may have to extend beyond 100 meters if at least 6 days has elapsed since *Aedes* mosquito could have fed upon viremic dengue cases.

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PREVALENCE OF *ANOPHELES* SPECIES AND THEIR INFECTION STATUS IN A MALARIA HYPOENDEMIC AREA OF RURAL BANDARBAN, BANGLADESH

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Bandarban, a district in the Chittagong Hill Tracts of Bangladesh, is hypoendemic for malaria. In 2009 an entomological study was begun to identify the malaria vectors, their population and infection dynamics year round during which a large scale malaria epidemiological study on more than 4,500 households with 20,000 people is ongoing. More than 95% of these households have at least one treated bednet. *Anopheles* mosquitoes were collected indoors with CDC miniature light-traps every month from selected houses in the study site. Each trap was deployed for at least 12 hours (6 pm to 6 am). After collection, mosquitoes were identified to species level using standard keys. ELISA was performed to detect *Plasmodium falciparum*, *P. vivax*-210, and *P. vivax*-247 circumsporozoite proteins (CSP). In total, 8,169 female *Anopheles* mosquitoes belonging to 22 species were collected from 1,619 trap-nights extending through December 2010. *An. philippinensis/nivipes* complex was the predominant species (25.3%), followed by *An. jeyporiensis* (16.8%) and *An. vagus* (14%). Seasonal variation existed in abundance of mosquito species. Ninety mosquitoes belonging to 13 species tested positive for *Plasmodium* infection, with an overall infection rate of 1.1% (90 of 8,061). The highest infection rate was found in *An. nigerrimus* complex (2.9%) followed by *An. maculatus* (2.7%) and *An. umbrosus* (2.4%). For the first time infections in *An. jeyporiensis* and *An. kochi* were documented in Bangladesh. Other important infected species were *An. baimaii*, *An. minimus s.l.*, *An. philippinensis/ nivipes* and *An. karwari*. In terms of density and incrimination, the *An. philippinensis/nivipes* complex*, An. jeyporiensis*, *An. vagus, An. nigerrimus* complex and *An. maculatus* seemed to play vital roles in malaria transmission in rural Bandarban. This study suggests that even in presence of insecticide impregnated bed-nets, a number of *Anopheles* species can still play a role in the transmission of malaria in Bangladesh.

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TOWARDS THE CONTROL OF CHIKUNGUNYA VECTOR IN LA REUNION USING THE STERILE INSECT TECHNIQUE: SEXUAL COMPETITIVENESS AND MATING SUCCESS OF STERILIZED MALES *AEDES ALBOPICTUS*

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The sterile insect technique (SIT) is widely used as part of area-wide integrated pest management programs for insects such as fruit flies, screw-worm flies and tsetse flies. It is based on the releases of large numbers of sterile males, which transfer their sterile sperm to wild females for the fertilization of the eggs, resulting in embryonic death. The lack of offspring could then lead to a decrease of the wild population density. The SIT is currently being developed at the FAO/IAEA in Vienna and on La Reunion, France, for the potential control of *Aedes albopictus*, vector of Chikungunya and secondary vector of Dengue viruses. In this context, we investigated the impact of the sterilisation process on male mating success and sexual competitiveness in the laboratory and under semi-field conditions. Males from a young laboratory colony (F4) were irradiated as pupae (>20h old) with gamma-rays at 35 or 40 Gy. In the laboratory, the time for male sexual maturation was not affected by the irradiation, neither was the insemination rate of 4 days old males as compared to un-irradiated ones. The daily individual mating success of sterile males during 15 days was reduced as compared to un-irradiated males; however the differences were significant only during the second week. Sterility was maintained over successive matings and after a resting period. Under semi-field conditions, sterile males competed well against wild males, though a resting period of 5 days in the laboratory before release greatly improved their efficiency. A ratio of five sterile to one wild male resulted in a two-fold reduction of the wild females' mean fertility. Valuable information on the effects of the sterilization process was collected and it is suggested that an efficient reduction of a wild population might be achieved by releasing sterile males as adults. Moreover, the results indicate that a resting period allows a recovery from radiation-induced somatic damages which improved the biological quality of the males.

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IMPACT OF HIGH COVERAGE WITH INSECTICIDE TREATED NETS ON MALARIA VECTOR TRANSMISSION INDICES IN SOUTH COAST KENYA

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In studies conducted during 1994-1998 on the south coast of Kenya, *Anopheles funestus* and *An. gambiae* s.s. were the primary malaria vectors, with *An. arabiensis* and *An. merus* playing a secondary role. To evaluate the impact of a recent upsurge in household bednet use in this area, the present study collected indoor resting malaria vectors in the same region using pyrethrum spray catches over a 21-mo period in 2009-2010. Species distribution of the recovered malaria vectors was determined, and house densities (mosquitoes/house) and human-biting rates (HBR) were estimated and compared with those reported in the same study area between 1994-1998, when bed net coverage was minimal. Present day bednet coverage and use were also determined in the houses where mosquito collections were conducted. In 2009-2011, the predominant malaria vectors in the study area were *An. funestus* and *An. gambiae* s.l., both of which are highly anthropophilic. A significant decline in the relative proportion of *An. gambiae* s.s. was observed, coupled with a proportionate increase of *An. arabiensis*. After 3-4 years of 60-86% coverage with ITNs, the density and human biting rate of indoor resting mosquitoes was estimated to have been reduced by more than 97% and 90% for *An. funestus* and *An. gambiae* s.l. respectively. The host feeding choice of both vectors shifted to non-human vertebrates, with an increase of 15% for *An. gambiae* s.l. and 5% for *An. funestus.* HBR was higher in houses without nets compared to houses with nets. This difference was significant for *An. funestus* but not for *An. gambiae* s.l. These entomological indices indicate a diminishing role of *An. gambiae* s.s. in malaria transmission in the study area due the high bed net coverage. While increasing bed coverage beyond the current levels may not significantly reduce the transmission potential for *An. arabiensis*, it is anticipated that increasing and sustaining high bed net coverage may result in a diminished role for *An. funestus* in malaria transmission.

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STABLE ISOTOPES INDICATE RESOURCE PARTITIONING AND PRIMARY FOOD RESOURCES OF MOSQUITO LARVAE IN WESTERN KENYA

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Larval density and inter-/intra-specific competition have been shown to have a significant effect on the development and growth rates of mosquito larvae. Food resource acquisition and the degree of resource partitioning among larvae sharing the same habitats are poorly understood; however, these are fundamental determinants of aquatic habitat productivity of malaria vectors in western Kenya. The current study uses $\delta^{13}C$ and $\delta^{15}N$ stable isotope analysis to evaluate resource partitioning and primary resources used by naturally occurring mosquito larvae in Iguhu, Kenya. Laboratory reared *Culex quinquifaciatus* and *Anopheles gambiae* s.s. fed with the same diet did not differ significantly from each other or the lab food ($F = 3.7270$, $P = 0.0662$ for $\delta^{13}C$ and $F = 0.0086$, $P = 0.9915$ for $\delta^{15}N$). Preliminary results from three natural habitats indicate that carbon isotope ratios were able to distinguish anopheline and culicine larvae, however nitrogen isotope ratios did not distinguish any difference (Rank sums test, $P = 0.0005$ for $\delta^{13}C$ and $P = 0.46$ for $\delta^{15}N$). Preliminary data suggests that isotopic ratios can also be able to distinguish among anopheline species sharing the same habitat. This work may provide insights into ecological control methods, such as using controphic species as bio-control tools. Controlled microcosm experiments and identification of relative food resource importance using natural stable isotopes are ongoing.

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MULTIPLEX XENOMONITORING OF *WUCHERERIA BANCROFTI* INFECTION AND *ANOPHELES PUNCTULATUS* SPECIES IN PAPUA NEW GUINEA

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The filarial parasite, *Wuchereria bancrofti* (Wb), causes lymphatic filariasis (LF) and is endemic to Papua New Guinea (PNG) with an estimated prevalence of >1 million individuals; *Anopheles punctulatus* sibling species are vectors of Wb. Currently LF elimination campaigns are underway in PNG and it will be important to monitor the progress of these efforts. Here we describe a high throughput post-PCR xeno-monitoring approach coupling an ITS2-based *Punctulatus* group identification method with detection of Wb DNA. Ligation detection reaction-fluorescent microsphere assay (LDR-FMA) strategies specifically differentiated the *Punctulatus* group species known to transmit Wb (*An. punctulatus, An. koliensis*, *An. farauti* s.s., *An. hinesorum* and *An. farauti 4*) and Wb (sensitivity = single microfilaria). Individual microfilaria were isolated from patient samples known to be Wb-positive by conventional parasitological diagnosis. Field-captured mosquitoes (n=1056) from five East Sepik villages were analyzed by the multiplex LDR-FMA. *Anopheles* species PCR amplification and LDR-FMA identification was successful for >90% of the collection, with *An. punctulatus* (AP) as the predominant species collected (n=752), followed by *An. koliensis* (AK) (n=195) and *An. hinesorum* (AH) (n=8). Wb DNA was detected in 19.1% of the collected specimens. Wb DNA was detected in 22% of mosquitoes identified as AP, 6% of AK and 50% of AH. This assay represents a useful xeno-diagnostic strategy for detecting the presence of Wb in specific species of the *Punctulatus* group. Because monitoring prevalence of Wb in the human population requires nighttime blood collections this assay provides an important alternative for evaluating LF-elimination progress. As malaria is co-endemic in PNG and transmitted by members of the *Punctulatus* group, future expansion of this assay seeks to include human *Plasmodium* species.

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A SPATIO-TEMPORAL BAYESIAN MODEL TO IMPROVE SURVEILLANCE AND CONTROL OF WNV VECTORS IN PIEDMONT, NORTHERN ITALY

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West Nile virus (WNV) first emerged in Italy in 1998 in an equine outbreak near the swamps of Padule di Fucecchio, Tuscany. No other cases were detected during the following decade, but there was evidence of continued virus circulation in the country. Between 2008 and 2010 outbreaks with a total of 18 cases of WNV in humans (4 of them fatal) and 70 in horses (19 fatal) occurred in 6 regions, spreading from their initial Northern foci throughout Italy. These outbreaks resulted in increased attention by public health authorities to the role of migratory and residential birds, and local mosquito vectors in virus dispersal and amplification. In order to identify areas with environmental conditions conducive for WNV amplification and transmission, we analyzed

longitudinal (2000-2006) mosquito data from CO_2-baited traps covering the Piedmont Region of Northern Italy, where migratory bird routes and suitable habitats for the vectors overlap. Weather data (temperature, rainfall and relative humidity) were collected from weather stations within the study area. Remote sensing imagery was employed for landscape characterization. We applied spatial statistics and a Bayesian Generalized Linear Mixed Model (GLMM) to: (a) describe the patterns of abundance and distribution of three putative WNV vectors *Ochlerotatus caspius, Culex pipiens* and *Cx. modestus*, and (b) predict the environmental conditions associated with their occurrence and spatial distribution. *Oc. caspius* and *Oc.caspius* were most abundant in rural areas, while *Cx. pipiens* near urban areas. Based on the best models we developed a prediction map of each vector species for the entire Piedmont Region, and predicted areas with the highest risk for WNV introduction and amplification. Our models show the importance of weather and environmental factors in predicting the abundance of mosquito abundance. More generally, our findings provide public health authorities with an useful surveillance tool that can be included in planning for vector in Piedmont and other regions of Northern Italy.

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CHANGES IN HETEROGENEITY AND INEQUALITY OF MALARIA RISK AFTER THE INTRODUCTION OF INSECTICIDE-TREATED BED NETS IN MACHA, ZAMBIA

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In 2007, insecticide-treated bed nets (ITNs) were introduced in Southern Zambia as a malaria control measure. To determine the effect of ITNs on heterogeneity in mosquito host choice, human microsatellite genotypes from blood fed mosquitoes were used to determine the proportion of multiple blood meals taken in a single gonotrophic cycle by *Anopheles arabiensis*, the primary malaria vector in the region. Compared to pre-ITN data, the multiple feeding rate dropped from 18.9% to 9.1%, suggesting that mosquito biting may have focused onto a smaller fraction of the population. To validate this hypothesis, unique human genotypes were identified from blood meals taken by mosquitoes in eight households before and after the introduction of ITNs. Pre-ITN, 20% of individuals in a household provided 40% of blood meals, which increased to 59% post-ITN. To measure heterogeneity over a larger scale, weekly mosquito collections were conducted in 90 households in two village areas over two months. In these collections, the top 20% of households contributed 73.9% of *An. arabiensis* mosquitoes, and 88.7% of blood fed *An. arabiensis*. Statistical analysis showed evidence that these high-risk households were spatially clustered. These data indicate that there is substantial heterogeneity in malaria risk both at the local and household level. This suggests that with additional tools, high risk areas can be identified and targeted for malaria control in order to more efficiently decrease transmission in an effort towards local elimination.

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SEASONAL VARIATION IN METABOLIC RATE AND BODY SIZE IN RELATION TO DRY-SEASON SURVIVAL OF *ANOPHELES GAMBIAE* IN THE SAHEL

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The African malaria mosquito, *Anopheles gambiae*, is distributed throughout sub-Saharan Africa, with much environmental variation across its range. Some populations inhabit areas without surface water for 3-8 months, yet whether populations persist over the long dry season by aestivation of adults or by migration from areas with permanent water soon after the first rains remains unknown. Recent studies suggest that M-form *An. gambiae* aestivate, however, the physiological and behavioral mechanisms by which they survive the dry season are still unknown. We undertook a year-long (October 2009-August 2010) study to measure seasonal variation in metabolism rate (MR) and body size, a key determinant of MR. Measurements were made prior to the dry season, throughout the dry season, and in the next wet season. Further, to assess specific differences between Sahelian populations and those near permanent water, similar measurements were made at another village (130 km away) near the Niger River. Significant and highly-seasonal variation in body size and MR was found in the Sahelian population, but not that located near the river. Body size of the M form increased from the wet season (Aug) to the mid dry season (Feb), but surprisingly fell before the end of the dry season (Apr-May). Notably, body size of the putative founder mosquitoes (appearing 1-2 weeks after the first rains, before new larvae could complete development) remained small. These seasonal differences in body size were observed in both females and males, suggesting two subpopulations of M form mosquitoes with distinct activity patterns exist during the dry season. Contrary to our *a priori* expectations, MR of the M form was higher in the dry season as compared with the wet (after accounting for assay temperature, flight activity, and body size). These results suggest that aestivating adults do not reduce, and possibly increase, their MR. However, we cannot rule out that these measurements pertain only to active, blood-seeking females collected indoors rather than to those hidden in their as-yet unknown shelters.

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DIVERSION EFFECTS FROM A SPATIAL REPELLENT APPROACH FOR DENGUE VECTOR CONTROL

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Aedes aegypti transmits dengue, an important viral disease of public health significance worldwide. Currently, there are no treatments for dengue and disease control relies on reducing vector populations. Control of *Ae. aegypti* has focused on source reduction of larval breeding sites or residual spraying of chemicals to kill adults. However, increasing rates and distribution of dengue cases world-wide indicate that other approaches to vector control are warranted. A proof-of-concept study is currently underway to evaluate the role of spatial repellency to reduce the density of host-seeking *Ae. aegypti* inside homes. The concept includes pushing the vector away from a house prior to entry in order to break man-vector contact using sub-lethal chemical doses. One debate surrounding this approach is whether or not repelled vectors will divert to untreated homes in the area at a greater rate than under conditions in which a spatial repellent is not used. If so, this may pose a greater risk of virus transmission to individuals in unprotected houses. Quantifying this behavior will provide insight into expected disease impact and necessary coverage rates of spatial repellent interventions. We report on *Ae. aegypti* diversion rates generated under field conditions using varying experimental hut mark-release-recapture study designs in both Thailand and Peru and provide comments on integrated approaches to mitigate risk to untreated spaces based on our results.

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OVERVIEW OF THE MALARIA TRANSMISSION CONSORTIUM RESEARCH PROJECT IN ZAMBIA

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Zambia is one of the six nations that the Malaria Transmission Consortium Project implementing various studies to develop tools for measuring malaria transmission intensity, assessing the effectiveness of combinations of malaria vector control interventions, and evaluate the impact of vector behaviour and insecticide resistance on the effectiveness of the control program. Entomological studies were carried out to evaluate mosquito sampling techniques for measuring malaria transmission intensity, and evaluate the impact of vector control interventions on the behaviour of malaria vectors. Our results have shown that light traps set beside to occupied mosquito nets could serve as alternative for conventional human landing catches for sampling the malaria vectors and estimating transmission intensity. Both *Anopheles funestus* and *Anopheles gambiae s.l* were equally predisposed to bite indoor or outdoor ($P = 0.529$ and $P = 0.0524$, respectively) regardless of vector control interventions in the area, and hence the need for additional vector control interventions to prevent residual transmission occurring outdoor and reduce mosquito population at source. Furthermore, large-scale longitudinal community based epidemiological and entomological studies are going on in fourteen clusters each with about 1000 people to determine the impact of combined use of indoor residual spraying and insecticide treated nets on the incidence of malaria and entomological inoculation rate. We are measuring monthly incidence through active and passive case detections as the main outcome measure and entomological inoculation rate as secondary outcome measure. The community cohorts are central in the implementation of large-scale epidemiological and entomological studies in the area.

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A SIMPLE AND EFFICIENT TOOL FOR TRAPPING OVIPOSITION SEEKING *ANOPHELES*

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No effective tool currently exists for trapping ovipositing malaria vectors. This creates a gap in our ability to investigate the behavior and ecology of gravid *Anopheles*. Here we describe a simple trap that collects ovipositing Anopheline and Culicine mosquitoes. It consists of an acetate sheet coated in glue that floats on the water surface. Ten breeding sites were selected in rural Tanzania and 10 sticky traps set in each. These caught a total of 74 gravid *Anopheles* (54 *An. arabiensis*, 1 *An. gambiae* s.s. and 16 unamplified) and 1333 gravid Culicines, in just two trap nights. This simple sampling tool provides an opportunity to further our understanding of the behavior and ecology of gravid female Anophelines. It strongly implies that at least two of the major vectors of malaria in Africa land on the water surface during the oviposition process, and demonstrates that Anophelines and Culicines often share the same breeding sites. This method has clear potential for the study of oviposition site choice and productivity, gravid dispersal, and vector control techniques which use oviposition behavior as a means of disseminating larvicides.

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COMPARING THE HUMORAL RESPONSE TO SAND FLY AND MOSQUITO SALIVARY PROTEINS IN INDIVIDUALS FROM AN AREA IN CENTRAL MALI

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Vector-borne diseases like malaria and cutaneous leishmaniasis (CL) are public health problems in several tropical and subtropical countries. Several reports have linked the presence of antibodies to arthropods salivary proteins and risk of vector-borne diseases. However, cross-reactivity between the antibodies to salivary proteins of blood-sucking arthropods has not been properly scrutinized. Here, we compared the humoral immune response to salivary proteins from blood-sucking arthropods in individuals living in Kemena and Sougoula, two villages located in central Mali, West Africa. Sand flies, mosquitoes and other blood-sucking arthropods are abundant in these villages. Therefore, individuals living in these dwellings are prone to bites of different blood-sucking arthropods during their lifetime. Firstly, we tested if individuals living in these villages had anti-saliva antibodies to *Phlebotomus duboscqi, Anopheles gambiae* and *Culex quinquifasciatus* (vectors of CL, malaria and filariasis, respectively). We measured IgG levels in the sera of 117 individuals between 2 to 92 years old by ELISA. We determined that most of these individuals produced antibodies to all the three species tested. To evaluate if antibodies specific to *P. duboscqi* would react against mosquito saliva, we compared the anti-mosquito saliva IgG levels before and after sera incubation with *P. duboscqi* saliva. Incubation of sera with *P. duboscqi* saliva had no effect on the levels of anti-mosquitoes saliva IgG. Moreover, we tested if incubation with *P. duboscqi* saliva would change the recognition pattern of mosquito salivary proteins by Western blot technique. We found that there was no alteration in the number of mosquito salivary proteins revealed before or after incubation with *P. duboscqi* saliva. Here, we suggest that there was no major cross-reactivity among *P. duboscqi and* mosquito saliva antibodies.

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ASSOCIATIONS BETWEEN LEVEL OF ANTI-*AEDES* SALIVA ANTIBODIES, PRESENCE OF MOSQUITO LARVA IN HOUSES AND SEVERITY OF DENGUE FEVER

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In November 2010 we performed a survey including 57 houses of three main cities in Norte de Santander (Colombia): Cucuta, Los Patios and Pamplona. Houses were examined for the presence of breeding sites positive for mosquito larvae, and blood was collected on filter paper from volunteers (n=97) after informed consent was given. Our survey found that the main breeding site was water tanks inside houses and that the main mosquito species was *Ae. aegypti*. In addition, following serological testing we found an association between the level of anti-*Ae. aegypti* saliva IgG antibodies and people living in houses positive for mosquito larvae. These levels were also higher in people with history of symptoms consistent with dengue fever than in people that did not report the disease. Interestingly, people with history of severe dengue presented significantly lower IgG antibody levels than people that have suffered dengue fever alone; although these levels were significantly higher than

those in people with no history of dengue. Our results suggest that the level of antibodies can be used as a proxy for mosquito bite exposure and a measure of dengue fever risk.

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EVALUATION OF PREDICTIVE MAPS FOR *AEDES AEGYPTI* LARVAL HABITATS IN TWO URBAN AREAS OF COSTA RICA

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The abundance of *Aedes aegypti* can be associated with urban structure and tree cover, which conceals and protects containers. The purpose of this study was to create and evaluate predictive maps for *Ae. aegypti* larval habitats in Puntarenas and Carpio, two very different urban environments in Costa Rica. Linear regression models for number of mosquito larval habitats had been developed for Puntarenas, and they showed a significant association with tree cover when corrected by the number of locations evaluated (R^2 = 0.650, p<0.001). Land cover maps were created from Quickbird satellite imagery of both sites. Data was extracted from 50 by 50 m cells, and parameters from the model were used to create predictive maps by determining the expected number of *Ae. aegypti* positive larval habitats in all cells that cover the urban areas. To evaluate maps, cells were randomly selected, and entomological evaluations were performed. Four categories were created for the number of larval habitats per cell: low (0-1), medium (2-3), high (4-5), and very high (6 or more). For both sites, the expected number of wet containers in sample cells fell within the 95% confidence interval of predicted values. In Puntarenas, 382 wet containers were identified, container index was 22.5% and Breteau index 43.7. Expected and observed categories of *Ae. aegypti* larval habitats per cell in Greater Puntarenas were significantly correlated (p=0,037). Only 32.5% of cells harbored the exact number of expected habitats, 74% contained the expected number +/- 2 habitats, and only 16% underestimated total larval habitats. In Carpio, 693 wet containers were identified, container index was 11.4% and Breteau Index 24.7. Expected and observed categories of *Ae. aegypti* positive habitats per cell were not significantly correlated in Carpio. Only 50% of cells contained the expected number +/- 2 habitats, and 29% underestimated the total observed. The most frequent *Ae. aegypti* larval habitats in Puntarenas included outdoor containers and miscellaneous objects, while larval habitats in Carpio were commonly human-filled, such as drums and buckets. These maps and models may be considered adequate for areas like Puntarenas, whereas they do not seem to apply for Carpio. Tree cover may provide useful information in sites where *Ae. aegypti* larval habitats include mostly outdoor rain-filled containers, as opposed to sites where containers are greatly affected by the need for water storage.

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CLIMATIC VARIABILITY AND LANDSCAPE HETEROGENEITY IMPACT URBAN MOSQUITO DIVERSITY AND VECTOR ABUNDANCE AND INFECTION

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Urban habitat heterogeneity can modify patterns of interactions across species and lead to spatially fine grained differences in β-diversity patterns and their associated ecosystem services. Here, we study the impacts of landscape heterogeneity and climatic variability on: (i) the richness and diversity patterns of mosquitoes (Diptera: Culicidae) and (ii) the abundance and West Nile virus infection rate of the house mosquito, Culex pipiens, in Chicago, USA. We conducted a four year long study (2005-2008) in 8 sites that captured a gradient of urban heterogeneities. We found a total of 19 mosquito species, a representative sample of mosquito species richness in the area, according to both model estimation (Chao2 ± S.E. = 20.50 ± 2.29) and faunal records for Chicago. We found that heterogeneity in the landscape was the best predictor of both mosquito species richness and diversity, with the most heterogeneous landscapes harboring the largest number of species. In general there were no changes in species richness over the years that could be associated with weather patterns and climatic variability (WPCV). In contrast, changes in diversity evenness showed signatures of WPCV. Our results also showed that WPCV had major impacts on house mosquito abundance and West Nile virus mosquito infection rate (MIR) patterns. Although MIR was independent of mosquito diversity, it was associated with overall mosquito abundance, which had a convex association with species richness (i.e., abundance increases to a point after which it decreases as function of species richness). Finally, our results highlight the importance of considering dominant vector species as part of a community of vectors, whose biodiversity patterns can directly or indirectly impact the risk of infectious disease transmission.

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NON-LINEAR IMPACTS OF CLIMATIC VARIABILITY ON *AEDES AEGYPTI* POPULATION REGULATION

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Aedes aegypti is one of the most common urban tropical mosquito species and an important vector of dengue, chikungunya, and yellow fever viruses. It is also an organism with a complex life history where larval stages are aquatic and adults are terrestrial. This ontogenetic niche shift could shape the density dependent regulation of this and other mosquito species because events that occur during the larval stages impact adult densities. Here, we present results from simple density-dependence mathematical models fitted using maximum likelihood methods to weekly time series data from Puerto Rico and Thailand. Density dependent regulation was strong in both populations. Analysis of climatic forcing indicated that populations were more sensitive to climatic variables with low kurtosis (i.e., climatic factors highly variable around the median) rainfall in Puerto Rico and temperature in Thailand. Changes in environmental variability appear to drive sharp changes in the abundance of mosquitoes. The identification of exogenous factors forcing the sharp changes in disease vector populations using their statistical properties, such as kurtosis, could be useful to assess the impacts of changing climate patterns on the transmission of vector-borne diseases.

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THE ROLE OF SWINE IN THE ECOLOGY OF JAPANESE ENCEPHALITIS VIRUS TRANSMISSION OF SOUTHERN VIETNAM

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Japanese encephalitis virus (JEV) is a mosquito-borne flavivirus disease of major public health importance and is endemic to both north and south Vietnam. Swine populations play a role in JEV transmission as both a reservoir and amplifying host. In general, infected adult pigs support transient but high titer viremias and remain asymptomatic. In contrast, naïve piglets exhibit fever and experience weight loss, and gilts or sows who become infected from 40-80 days of gestation often abort or give birth to stillborn mummified fetuses. In some JEV-endemic countries, swine vaccination is performed within commercial livestock sector to prevent losses in reproductive performance of breeding herds. Here we review previous unpublished studies of JEV seroprevalence

within Vietnamese swine from the 1970s and 1990s, as well as novel data generated in our lab on seroprevalence of swine from southern Vietnam from 2006-2010. We discuss the potential utility of measuring JEV seroconversion in sentinel pigs for monitoring virus circulation in nature, and geographic targeting of childhood vaccination strategies. Furthermore, the swine seroprevalence data are used as input parameters for models of disease transmission dynamics, to examine the relationships between hyperendemicity, herd immunity, and the emergence of symptomatic disease.

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CLUSTER RANDOMIZED CONTROLLED TRIAL TO DETERMINE THE ADDITIONAL BENEFITS OF TOPICAL REPELLENTS TO LONG LASTING INSECTICIDE NETS (LLINS) ON MALARIA INCIDENCE

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Long lasting insecticide nets (LLINs) is the most effective tool against malaria vectors. Extensive LLINs coverage may give communal protection to non-users, by reducing vector populations or diverting them to other hosts. On the other hand LLINs may force mosquitoes to feed earlier in the evening and thus reducing the efficacy of LLINs or even divert mosquitoes to non-users, putting them at a higher risk of malaria. Several studies have shown significant malaria reduction in groups that use LLINs plus repellents versus LLINs only in areas where early evening malarial vector exposure occurs. This study tested whether repellents, used in combination with LLINs, can provide additional protection from malaria in an area of high LLINs use and early evening exposure in rural Tanzania. Data on the efficacy of deet (N, N-Di ethyl-3-methyl-benzamide) repellent against mosquito bites was collected in semi-field trials and field trials carried out in rural Tanzania. A 3 × 3 Latin square was used in both these trials and data was analyzed using Generalized estimating equations (GEE). Data on clinical outcomes was collected using a blinded cluster-randomized controlled trial measuring malaria incidence by passive case-detection in the intervention arm, (LLINs plus15% topical deet repellent) and control arm (LLINs plus placebo lotion). The trial was designed to have 80% power, to detect a 24% treatment effect at 95% confidence interval and a correlation co-efficient of 0.25. Compliance was measured using questionnaires. Malaria was tested using rapid diagnostic tests at a local clinic. Clinical malaria incidence rates, measured in person years were the outcome. Socio-economic parameters were measured by PCA. The results of the efficacy trials show that 15% deet repellent is 90% protective against all mosquito species under field conditions, and 70% protective against high biting density of *An. gambiae* in semi-field conditions over a period of 4 hours. Clinical data demonstrated a reduction of malaria in the intervention arm, though this difference was not significant.

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ENTOMOLOGICAL MONITORING AND EVALUATION OF INDOOR RESIDUAL SPRAYING IN UGANDA

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Malaria prevalence is high in Northern Uganda particularly in children under five years of age where *Anopheles gambiae s.s.* and *An. funestus* are the main vectors. To bring malaria under control, a large scale indoor residual spraying (IRS) program with Bendiocarb 80% wettable powder at 0.4 g/m2 was conducted from June 2010 to March 2011in six highly malaria endemic districts. During this period, two consecutive spray cycles were conducted in each district, with 5-6 months intervals. Pyrethrum spray catches (PSCs) were performed two to three weeks prior to and after IRS in 18 sentinel sites (12 houses /site) to assess the impact of IRS on vector indoor resting densities. World Health Organization wall cone bioassays were also conducted to measure the quality of spraying immediately after IRS, and at monthly intervals thereafter to track the residual efficacy of the insecticide on sprayed surfaces following the spraying. The PSC result shows that spraying Bendiocarb significantly reduced vector indoor resting densities in all households in both spray rounds. In round one, pre and post IRS-indoor resting densities were 12.33 and 0.097per house respectively ($p<0.001$). In round two, pre-IRS indoor densities were still low, 0.305 per house after 4-5 months following round one and reduced to 0.0972 per house thereafter in round two post IRS($p<0.001$), suggesting that the subsequent spraying of Bendiocarb at 5-6 month intervals further reduced the vector densities. The bioassay tests conducted soon after IRS showed 100% knock-down (KD) rates and 24-hour mortality in all tested houses, indicating high quality IRS. The monthly bioassays revealed that the insecticidal effect of Bendiocarb remained at 100% for three month period and dropped to 80% (24 hour mortality) in the fourth month after IRS. This study suggests that entomological monitoring to measure the impact of indoor residual spraying on vector population is useful and routine PSC as well as cone bioassays could also be used as an operational tool for programmatic decisions in IRS programs.

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EVALUATION OF ZEROVECTOR® DURABLE LINING (DL) - IMPACT ON *AEDES AEGYPTI* AND *ANOPHELES STEPHENSI* UNDER VARYING DL COVERAGE

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Recently, a Durable Activated Residual Textile (DART) product has been developed as an alternative to the traditional indoor residual spray (IRS) strategy. The function of this durable-lining (DL) material is to transfer toxic doses of deltamethrin to vectors resting on the surface of the material placed along the interior walls of homes thereby reducing overall populations and biting pressures to human hosts. Our previous studies focused on the resting, escape and mortality behavior of non-bloodfed (i.e. host-seeking) *Ae. aegypti* test cohorts using varying coverage levels of DL material to describe the negative effects of "safe-sites" (areas of the wall where DL is not present) on overall efficacy. Here we present results on continued studies describing the resting preference, knock down (KD) and 24 h mortality responses of bloodfed *Ae. aegypti* and unfed *Anopheles* stephensi females exposed to varying surface area coverage of DL (100, 75, 50 and 25%) under laboratory conditions. Studies evaluating the DL color preference of *Ae. aegypti* against blue and green DL were also performed. For all assays in which treated DL was applied, there was less resting response overall - even on safe sites (i.e., metal surfaces) within the assay device- and significant increases in the proportion of test cohorts flying and exhibiting KD compared to matched controls. This indicates an agitation response from the chemical active that was true even at a 25% coverage ratio. Mortality rates at 24h post-exposure from resting preference studies and KD rates from escape response assays indicate the DL product delivered a toxic dose of insecticide to both bloodfed *Ae. aegypti* and unfed *An. stephensi* assay populations at all surface coverage ratios. Overall, there was no significant DL color preference (either green or blue) observed under these test conditions. Combined, these results suggest minimal negative effects of color or safe sites to the overall efficacy goals of the DL product for both a dengue and malaria vectors. Similar studies will be repeated against bloodfed anopheline test cohorts, with field validation of laboratory findings currently in preparation.

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STABLE ISOTOPE ANALYSIS OF *AEDES AEGYPTI* AND *CULEX QUINQUEFASCIATUS* REVEALS LARVAL HABITAT SOURCES OF ADULT MOSQUITOES IN PUERTO RICO

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Larval habitats of the dengue virus vector, *Aedes aegypti*, are diverse and include ones above ground (such as water-filled pails, pails with water and plants, and discarded tires) and below ground (such as septic tanks). Quantifying which larval habitats contribute to the bulk of the adult vector population has proven difficult but is important to understand the basis for mosquito production and resultant virus transmission, and to design habitat-specific vector control programs. Stable isotope analysis provides a tool for determining the food resource base of mosquito larvae by evaluating habitat-specific 13C:12C and 15N:14N ratios. These habitat-specific ratios are retained in tissue of emergent adults; therefore, individual adults can be assigned to original larval habitats. In this study, we analyzed carbon and nitrogen stable isotope ratios of adult *Ae. aegypti* and *Culex quinquefasciatus* that had emerged from a diverse array of habitats in dengue-epidemic prone communities of southern Puerto Rico. Multiple logistic regression revealed habitat specific signatures and specifically separated below-ground (septic tank) and above-ground habitats for male and female adults of both species. Further, discarded tires were separable from water-filled pails and from pails with plants, all above-ground sources. Carbon ratios were particularly predictive as adults from septic tanks were enriched with 13C compared to adults from above-ground sources. Nitrogen isotope ratios were less predictive except that adults that had emerged from discarded tires had a higher 15N enrichment compared to other habitats. Carbon:nitrogen ratio in *Ae. aegypti* was higher than in *Cx. quinquefasciatus*, probably because the former species better withstands nitrogen limiting conditions. Overall, the carbon and nitrogen stable isotope dynamics likely reflect different nutrient inputs, microbial metabolic processes, and decomposition pathways operating in these different larval environments.

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DYNAMIC COMPONENTS OF THE MATING SYSTEM OF THE DENGUE VECTOR *AEDES AEGYPTI*

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Dengue is a major cause of hospitalization and death among children in Southeast Asia. Dengue virus is transmitted through the bite of infected *Aedes aegypti* females. There are approximately 50 million people infected by dengue annually (WHO 2010). In order to control this disease, we need to control mosquito populations. Understanding mosquito mating behavior and frequency, including male preference for females is an important key affecting mosquito population structure and genetic control efforts. We investigated the frequency of multiple mating and more specifically, the frequency of sperm transfer and female utilization of sperm from more than one mating. In this study, we report our results of female sperm usage patterns using a combination of approaches including PCR-based detection of sperm genotypes and screening of female reproductive output. In addition, little information is known about the preferences of free-ranging wild male *Ae. aegypti* for female body size. Mosquito mating patterns are a fundamental component of sexual selection and may have significant influences on the genetics and demographics of mosquito populations.

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COMPARATIVE EVALUATION OF ALTERNATIVE MOSQUITO SAMPLING METHODS IN LOWLAND SOUTHEAST, ZAMBIA

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Sampling malaria vectors is paramount for measuring malaria transmission. Human landing catch (HLC) has been the gold standard over the years but due to potential infective bites, attempts to develop alternative mosquito sampling methods have been made in malaria endemic countries. Centre for Disease Control and prevention miniature light trap (CDC-LT), Ifakara Tent Trap design model C (ITT-C), HLC outdoor, standardized resting boxes (RBO) and exit window traps (EWT) were evaluated against the standard HLC indoors in south east of lower Zambia. A 3 X 3 Latin square design was used with a series of ten rounds. Generalized Linear Model was applied to estimate mean catches to determine their efficiency and binary logistics to determine proportionality of abdominal status per sampling method. CDC-LT was 1.459 and 1.134 more sensitive than human landing catches indoor for sampling *Anopheles gambiae sensu lato* (s.l) (Giles) and *An. funestus* (Giles) species respectively. HLC outdoor, ITT-C had relative sensitivity (RS) of 0.594 and 0.040 for sampling *An. gambiae s.l* and 0.929 and 0.608 for *An. funestus* respectively. ITT-C collected more *An. funestus* than other mosquito species. Resting boxes and the exit window traps were the least efficient sampling methods. Proportionally 28.6% and 14.1% of blood fed *An. gambiae s.l* and *An. funestus* were collected respectively in ITT-C a tool supposedly to be exposure free. CDC-LT was the most efficient sampling method in the study site but routine monitoring of programmes may pose challenges ranging from availability of batteries and skilled manpower.

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BIOLOGICALLY MEANINGFUL COVERAGE INDICATORS FOR MALARIA VECTOR CONTROL INTERVENTIONS

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Indoor residual spraying (IRS) and insecticide-treated nets (ITNs) have been shown to dramatically reduce malaria transmission but cannot completely eliminate it from most settings in Africa. Although, mosquito species which predominantly feed on animals transmit malaria less efficiently, they are primary malaria vectors in many tropical countries and can dominate residual transmission in Africa where ITNs or IRS are widely used. While ITNs are known to confer personal protection against any mosquitoes attempting to bite while they are in use, it remains unclear whether they confer community-level protection for predominantly zoophagic vectors. Here we use a process-explicit malaria transmission model to assess the likely extent and mechanism of community-level impact of ITNs upon human malaria exposure to zoophagic vectors. Consistent with field observations in Africa, ITNs are most effective against anthropophagic vectors because the fraction of available blood that covered people represent is very high so that survival per feeding cycle is reduced, the length of feeding cycle is extended, and the emergence rate for adult mosquitoes is reduced. ITNs are less effective against zoophagic vectors because animals are available as alternative blood sources so negligible impact upon survival, feeding cycle length or reproduction rates occurs. Nevertheless, ITNs can deliver appreciable communal protection against transmission by zoophagic vectors so long as exposure predominantly occurs indoors. This is because the very small proportion of a zoophagic

vector population that get killed or diverted by ITN are the same proportion that actually transmit malaria so reducing an already low proportion of bloodmeals taken from humans has a substantial impact upon overall transmission. We also examine what coverage and protection really mean and how they determine the impact on real-world public health programmes. Further reduction of malaria transmission will require new methods for measuring and targeting blood resources other than indoor-resting humans, which mosquitoes depend upon for survival.

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TARGET PRODUCT PROFILE CHOICES FOR INTRA-DOMICILIARY MALARIA VECTOR CONTROL PESTICIDE PRODUCTS: REPEL OR KILL?

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The most common pesticide products for controlling malaria-transmitting mosquitoes combine two distinct modes of action: 1) conventional insecticidal activity which kills mosquitoes exposed to the pesticide and 2) deterrence of mosquitoes away from protected humans. While deterrence enhances personal or household protection of long-lasting insecticidal nets and indoor residual sprays, it may also attenuate or even reverse communal protection if it diverts mosquitoes to non-users rather than killing them outright. Here we describe a process-explicit model of malaria transmission which captures the sequential interaction between deterrent and toxic actions of vector control pesticides and accounts for the distinctive impacts of toxic activities which kill mosquitoes before or after they have fed upon the occupant of a covered house or sleeping space. Increasing deterrency of intradomiciary measures such as indoor residual spraying (IRS) and insecticide-treated nets (ITNs) increases personal protection but consistently reduces communal protection because deterrent sub-lethal exposure inevitably reduces the proportion subsequently exposed to higher lethal doses. If the high coverage targets of the World Health Organization are achieved, purely toxic products with no deterrence are predicted to generally provide superior protection to non-users and even users, especially where vectors feed exclusively on humans and a substantial amount of transmission occurs outdoors. Remarkably, this is even the case if that product confers no personal protection and only kills mosquitoes after they have fed. Products with purely mosquito-toxic profiles may therefore be preferable for ITN or IRS programmes with universal coverage targets, rather than those with equivalent toxicity but which also have higher deterrence. However, if purely mosquito-toxic products confer little personal protection, then they will require aggressive "catch up" campaigns, with behaviour change communication strategies that emphasize the communal nature of protection, to achieve high coverage rapidly.

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TUBERCULOSIS IN NINGXIA HUI AUTONOMOUS REGION, THE PEOPLE'S REPUBLIC OF CHINA

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Tuberculosis (TB) is a major cause of morbidity and mortality throughout the world. Practical control relies on rapid and effective diagnosis and treatment of active cases in order to protect vulnerable subjects and decrease transmission. China is the second most endemic country for TB, after India. Ningxia Hui Autonomous Region (NHAR), located in the north-west, is one of the poorest areas in China and national surveys have revealed a very high prevalence of human TB. A retrospective TB study we undertook of TB clinical records from NHAR further disclosed the serious nature of the infection there. The numbers of advanced TB cases were highest in two counties - Xiji and Guyuan with prevalences of 16.7% and 18.5%, respectively. More than 85% of active cases in Xiji County undertook treatment for over 12 months, although the remainder of the prolonged treatment cases appeared to correlate with neither disease stage nor disease incidence. Haiyuan and Tongxin counties had the highest incidence with the lowest population densities and the largest areas suggesting that they might account for the majority of TB transmission. On average between 10.9%-15.3% of NHAR patients were not diagnosed for 6 months in 2005-2009. This is of great public health concern and may account for increased TB transmission in these areas. The distribution of sputum-positive cases showed the lack of correlation with disease stage or incidence, which again suggests that there may be important epidemiological and socio-economic factors at play. From these data, we can conclude that there is a lack of community TB awareness and, in part, poor awareness and/or training in local health facilities which directly increases community exposure and fuels TB transmission. The data also showed a significant failure of DOTS which may reflect poor compliance. This again unavoidably promotes TB spread and will undoubtedly escalate the TB disease burden in future. Given the increase in population migration, the threat of TB is critical not only for NHAR and other parts of China but globally.

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EVIDENCE OF CROSS-REACTIVITY AGAINST AVIAN H5N1 AND PANDEMIC H1N1 2009 INFLUENZA VIRUSES FOLLOWING VACCINATION WITH A PRIME-BOOST REGIMEN OF SEASONAL INFLUENZA VACCINES

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Recent studies have demonstrated that inactivated seasonal influenza vaccines may elicit production of heterosubtypic antibodies, which can neutralize avian influenza H5N1 virus in a small proportion of subjects. We hypothesized that administration of a prime boost regimen of live and inactivated seasonal influenza vaccines would enhance the production of heterosubtypic antibodies and provide evidence of cross-protection against a range of influenza viruses. In a randomized open-label pilot feasibility study, 26 healthy adult volunteers were randomized to receive 1 of 4 vaccine regimens containing two doses of 2009-10 seasonal influenza vaccines administered 7 weeks apart. Group (1) received 2 doses of live attenuated intranasal influenza vaccine (LAIV); Group (2) received 2 doses of inactivated influenza vaccine (IIV); Group (3) received LAIV then IIV; Group (4) received IIV then LAIV. A range of assays for avian H5N1, 2009 pandemic H1N1, and seasonal vaccine influenza strains were performed on blood and nasal wash samples collected pre-vaccine and 2 and 4 weeks after each dose, and the percentage of cytokine-producing cells 14 days after each dose was compared with baseline. As expected, subjects receiving IIV had prompt serological responses to vaccine strains, which were not measureable in subjects receiving intranasal vaccine. Two of 8 subjects in Group 3 demonstrated cross-reactivity against H5N1 and pandemic H1N1 2009, as well as against the seasonal vaccine strains. All vaccine regimens were safe and well tolerated. In this pilot study a prime-boost regimen of seasonal influenza vaccines gave laboratory evidence of cross-protection against both avian and pandemic influenza in a quarter of subjects. This strategy may be a useful adjunct in the event of a new

influenza pandemic while a specific vaccine is being developed. Further work is needed to study the immune response to influenza vaccines in the expectation that a universal influenza vaccine can eventually be developed.

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HUMAN INFLUENZA SENTINEL SURVEILLANCE IN REMOTE BORDER POPULATIONS IN WESTERN CAMBODIA

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Seasonal, pandemic H1N1 and avian influenza viruses have been reported from Cambodia. However, despite significant cross-border population movement, influenza surveillance data on the Cambodian side of the Thai-Cambodian border is limited. In May 2010, the Armed Forces Research Institute of Medical Sciences (AFRIMS) initiated an influenza sentinel surveillance study in Western Cambodia with the Cambodian Communicable Disease Control (CDC) Department and Institut Pasteur du Cambodge. Epidemiological data and nasal and throat specimens were collected from outpatients who presented with influenza-like-illness (ILI - fever > 38°C and cough or sore throat) at sentinel health facilities in Battambang and Oddar Meanchey provinces. Real-time PCR was performed to distinguish influenza A and B, and positive samples were subtyped. Influenza-negative samples were analyzed for alternate respiratory pathogens by inoculation onto MDCK cells and detection by immunofluorescence test. Of 82 ILI patients recruited between May 2010 and May 2011, 13 specimens tested positive for influenza by real-time RT-PCR between August and October 2010, of which 12 were influenza A and 1 influenza B. Subtyping of influenza A viruses detected 9 (75%) pandemic influenza A/H1N1(2009) and 3 (25%) influenza A/H3N2, yet no influenza A/H5N1 or seasonal influenza A/H1N1. Among flu-negative samples tested to date, only 1 non-influenza respiratory pathogen (parainfluenzavirus type 3) was detected. Genomic data, along with oseltamivir susceptibility data from influenza A isolates will be presented. In newly established sentinel surveillance sites along the Thai-Cambodia border, influenza virus was relatively infrequent among patients presenting with ILI, with pandemic influenza A/H1N1(2009) being the most common virus identified. Highly Pathogenic Avian Influenza (HPAI) virus H5N1 was not detected, and few common respiratory pathogens were isolated from influenza-negative samples.

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SEASONAL LEVELS OF INDOOR RESPIRABLE PARTICULATE MATTER IN A LOW-INCOME COMMUNITY IN URBAN BANGLADESH

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Acute lower respiratory infections (ALRI) are the leading cause of death in children <5 years of age in Bangladesh and indoor exposure to respirable particulate matter has been repeatedly associated with increased risk of ALRI in young children. This study aimed to describe air pollution exposures in households with young children in urban Dhaka and seasonal variation of this exposure. Two hundred and thirty-five households with children aged <18 months in an urban, low-income neighborhood were enrolled. Particulate matter approximately 2.5 microns in diameter ($PM_{2.5}$) was measured in the child's sleeping space for one 24-hour period each month using a portable monitoring device during May 2009 - April 2010. We calculated the arithmetic mean concentration of $PM_{2.5}$ among all households by month and investigated differences in mean concentrations by the type of cookstove used. We compared these values to WHO recommended daily mean values of 25 μg/m^3. We estimated the average number of hours per day that $PM_{2.5}$ levels exceeded 100, 250, 500, and 1000 μg/m^3. Seventeen percent of 235 households reported burning biomass as their primary cooking fuel; all other households cooked with natural gas or electricity. Mean $PM_{2.5}$ concentrations were 200 μg/m^3 but were significantly higher in households that burned biomass compared to cleaner fuels (265 vs 187μg/m^3, p=0.004). Biomass users had higher concentrations during all seasons. The highest mean concentrations were observed during winter for both biomass and cleaner fuel users (467 and 363 μg/m^3). Overall, $PM_{2.5}$ concentrations were above 100 μg/m^3 for approximately 5 hours and 30 minutes per day, but this increased to >10 hours per day during the winter. The hours that concentrations exceeded 250, 500 and 1000 μg/m^3 all doubled during winter compared to the yearly average. $PM_{2.5}$ concentrations exceeded 1000 μg/m^3 for more than an hour a day during the winter. Our study suggests that 24-hour mean $PM_{2.5}$ concentrations are frequently 10 times higher than the WHO recommended 24-hour mean in this low-income urban neighborhood and may contribute to increased risk of ALRI for children in this community. Burning biomass for cooking was associated with significantly higher $PM_{2.5}$ concentrations but even households that burned cleaner fuels were highly polluted, particularly during the winter. In this setting, high indoor $PM_{2.5}$ concentrations are not fully explained by burning biomass.

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DELAY IN DIAGNOSIS OF TB PATIENTS IN ADEN GOVERNORATE, YEMEN: EVALUATION OF THE HEALTH SECTOR PERFORMANCE

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Yemen has adopted the DOTS strategy to control TB since it was launched by the WHO STOP TB initiative in 1995. The program functions under the supervision of the public health sector without involvement of the private sector. Aside from being not involved, doctors in the private sector may also resist to follow the WHO's DOTS guidelines which have been proved highly effective in managing sputum-smear positive (SS+) TB patients. We designed a retrospective cross-sectional study to compare the delay in diagnosis and treatment of SS+ TB patients between the public and private health sectors. A total of 171 new SS+ patients aged 15 years or older registered in Aden governorate over a three-quarter period (Oct, 2008-June, 2009) have been interviewed using a pre-designed and tested semi-structured questionnaire to collect the required data including patient's care-seeking behavior. Written informed consents have been obtained from all patients who were willing to participate; data has been analyzed using SPSS 18.0 and STATA v9. 112 (65.5%) males vs. 59 (34.5%) females with a male to female ratio of 1.9:1, mean age of 35.9 (±15.18) years, 161(94.2%) were Yemeni nationals vs. 9 (5.8%) Somalian and 1 (0.6%) Ethiopian refugees. 146 (85.4%) patients were residents in Aden vs. 25 (14.6%) from other governorates, 23 (13.5%) patients had DM and 62 (36.3%) patients had a positive family history of TB. Mean patient delay (appearance of symptoms until seeking care) was 36.2(±67.58) vs. 37.24(±123.4) days; mean diagnostic delay (patient's first contact with the health provider until TB diagnosis) was 9.8 (±15) vs. 19.45(±42) days; and a mean treatment delay (diagnosis until start of treatment) of 1.56(±1.6) vs. 1.56(±0.86) days for public and private sectors respectively. After logarithmic transformation of the three means, only the mean diagnostic

delay was significantly longer in the private sector than in the public sector (one-way ANOVA, F= 5.11, p<0.02). Not only emphasizing the importance of adhering to the WHO guidelines for DOTS strategy; these findings necessitate the urgent integration of the private health sector into the DOTS strategy to reduce the gap between the public and private health sectors and to positively strengthen the activities directed to control TB in Yemen.

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MOLECULAR ANTIVIRAL SUSCEPTIBILITY TESTING OF SEASONAL INFLUENZA A VIRUS ISOLATES OBTAINED IN KENYA IN THE YEAR 2008-2009

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Antivirals play an important role in treatment and prevention of severe influenza infections. Amantidine and remantidine inhibit M2 protein of influenza A while oseltamivir and zanamivir inhibit NA of influenza A and B. M2 protein mediates influx of protons through the viral lipid membrane causing dissociation of the virus during virus entry. Binding of M2 inhibitors to M2 inhibits viral genome uncoating and RNP import into the nucleus. NA catalyzes removal of terminal sialic acid residues from viral and cellular glycoconjugates to facilitate virus release. NA also helps virus spread through circulation by removing sialic acids from cell surfaces. NA inhibitors interfere with release of progeny virus from infected cells preventing infection of new cells and halting the spread of infection. Mutations in M2 and NA proteins underpin these resistances at the molecular level. H274Y (H275 in NA1) change in the NA protein alters drug binding resulting in oseltamivir resistance. S31N substitution in the M2 domain determines antiviral resistance to M2 inhibitors. We investigated genetic characteristics of NA and M2 genes of seasonal influenza A virus isolated in Kenya in 2008-2009 in relation to antiviral resistance. Nasopharyngeal specimen from out patients ≥ 2 months old were screened by rtRT-PCR. Positive specimens were inoculated on MDCK cells followed by RNA extraction and amplification of M and NA genes. We sequenced 12 influenza A(H1N1) and 36 influenza A(H3N2) M and NA genes. 58% of influenza A(H1N1) viruses had H275Y mutation but none had S31N change. All H3N2 strains had the S31N mutation in M2 protein. All H3N2 strains lacked H274Y mutation. These results conform to the global picture regarding influenza antiviral activity during the period. In conclusion, genotypic data obtained here demonstrate antiviral resistance in seasonal influenza A viruses isolated in Kenya in 2008-2009 despite lack of widespread antiviral use. Our results emphasize the unpredictable nature of influenza viruses and need for continued surveillance of drug resistance patterns globally.

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A QUALITY ASSESSMENT TOOL FOR TUBERCULOSIS CONTROL ACTIVITIES IN RESOURCE LIMITED SETTINGS

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Tuberculosis (TB) is a significant problem, infecting nearly 9 million new patients per year and killing about 2 million a year. The primary means with which to affect TB globally are to decrease transmission locally, mainly by effective identification, diagnosis, and treatment of infectious TB patients. Therefore, quality assurance of TB control efforts at the local level is essential. This study describes the creation of a data extraction tool for retrospective chart review based on the *International Standards for TB Care, 2009* for the assessment of TB control programs located in resource limited settings. The tool was field tested at a rural mission hospital in central Kenya. Results highlight multiple areas of excellence in TB care such as patient retention, treatment completion, and care of HIV/TB co-infected patients. Quality improvement interventions might best be focused on smear negative diagnosis and follow up sputum smears for smear positive patients. The process prompted revision of the tool to clarify questions and answers. The final product is a good tool to collect data for use in quality assessment and improvement of local TB control programs in resource limited settings.

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GENOME-WIDE ASSOCIATION STUDIES OF TUBERCULOSIS AND LEPROSY SUSCEPTIBILITY IDENTIFY COMMON PATHWAYS INVOLVED IN BOTH DISEASES

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Tuberculosis (TB) and leprosy cause significant worldwide morbidity and mortality, predominately in resource poor settings across Africa and Southeast Asia. Although TB and leprosy represent the largest burden of mycobacterial disease in humans, relatively few studies have attempted to analyze human susceptibility loci which associate with predisposition to both of these diseases. TB and leprosy are significantly divergent in terms of phenotypic presentation, however, the causative agents *Mycobacterium tuberculosis* (TB) and *Mycobacterium leprae* (leprosy) share a common origin, and it is likely that the human innate immune system detects these pathogens using shared Toll-like receptor signaling. Our group has recently published genome-wide studies on both tuberculosis and leprosy and we here identify genes and pathways that associate with human genetic susceptibility to both diseases using data from these genome-wide studies. Aggregate gene based association statistic for mycobacterial susceptibility was computed using data from tuberculosis and leprosy susceptibility studies and these results were further carried forward for pathway analysis. Significant associations were observed between the *CYP11B1/2*, *CTSB/FDFT1*, *CYP26A1*, *AGER* and susceptibility to common mycobacterial diseases (P_{best}=5.1x10^{-6}). Pathway analysis implicates the lipid and steroid hormone metabolism pathways (P_{best}=2.2x10^{-5}). In summary, we find evidence that lipid and steroid hormone metabolism pathways play an important role in susceptibility to both tuberculosis and leprosy. We are now initiating a genome-wide study of Buruli ulcer (BU), caused by *Mycobacterium ulcercans*, in African populations to compare overlapping susceptibility loci between these three mycobacterial diseases. These studies have the power to elucidate novel molecular pathways which represent potential targets for pharmacological intervention for future treatment of mycobacterial diseases.

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TOWARDS GLOBAL CHARACTERIZATION OF ENVIRONMENTAL AND CLIMATIC DETERMINANTS FOR SEASONAL INFLUENZA

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Recent experimental studies have established the role of factors such as humidity and temperature in influenza transmission. Historically, spatiotemporal spread of influenza has been observed to follow a

latitudinal gradient, with environmental and climatic factors. With the capability of pandemic and seasonal influenza to spread rapidly worldwide - it is even more important to understand how climactic and environmental factors affect the efficiency of influenza transmission in different parts of the world so as to enhance multilateral efforts for prevention and control. We use NASA satellite-derived data in various population centers throughout the world to obtain indicators including land surface temperature and precipitation, and ground station measurements such as relative humidity. Trends of influenza-like illnesses in these same population centers were also identified. We further developed linear and non-linear models, in order to determine the dominant factors contributing to influenza transmission. Since different countries have varying completeness of data and different surveillance systems, we used a range of models, including time series regression and neural network. The first phase of our study included countries in North and Central America, and Northern Europe, encompassing both sub-tropical and temperate climate. Our results show that measures for rainfall, temperature, relative humidity and solar radiation contribute to influenza dynamics. About 60% of influenza variability in temperate regions can be accounted by these factors. Whereas in sub-tropical region, previous number of influenza cases are additionally needed as a determinant. Our best fit models can predict influenza cases with more than 75% accuracy. Our study may help develop better ability to forecast influenza activity worldwide. The methods in turn, can be integrated into surveillance system as an early warning system, for both seasonal and pandemic influenza.

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BORDER CONTROL MEASURES AND TERRITORIAL SURVEILLANCE IN AMERICAN SAMOA FOR THE 2009 H1N1 INFLUENZA PANDEMIC

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Our objective is to describe the disease prevention, case identification, and treatment approaches of the American Samoan Unified Health Command during phase one of the 2009 H1N1 Influenza Pandemic. A retrospective review of public health surveillance records, hospital infection control records, and event after-action reports was completed. Descriptive statistics were used to evaluate data. Border surveillance measures were in effect from May 3 to June 8 when the first confirmed H1N1 case was documented in the territory. Health officials met all incoming aircraft and sea vessels at territory ports of entry. Surveillance forms were requested from each passenger, and passive screening techniques for illness identification were employed. Cases of influenza-like-illness were further investigated at a local clinic and suspected travelers were placed in community isolation. Greater than 3200 man hours were documented during surveillance activities. Hospital surveillance data from May 3 to July 31, 2009 was collected. Ninety-nine influenza swabs sets were collected. On sight rapid testing demonstrated 19.2% (n=19) Influenza A + samples, and 37.4% (n=37) H1N1+ samples by confirmatory testing off-island. The sensitivity and specificity of this screening strategy were 30.5% and 87.3% respectively (PPV 57.9%, NPV 68.7%). A true positive rate of 11.1% and false negative rate 25.3% further complicated medical decision-making based on rapid testing alone. Eight percent of cases were suspected concomitant seasonal influenza A strains. No fatalities were reported. In conclusion, a unified health command structure was effective in responding to this emergency. Response planning was appropriate and rapidly implemented. Border control surveillance efforts were largely ineffective. Case identification and medical treatment protocols were hindered by remote locale. Despite the challenges discussed, no fatalities were reported.

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AN EIGHT ANTIGEN MULTIPLEX ELISA DERIVED FROM A FULL PROTEOME SCREEN FOR ACCURATELY DETECTING ACTIVE TUBERCULOSIS

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Developing accurate serological assays for the detection of active tuberculosis has been difficult, because no single antigen has been found that is recognized by serum antibodies in every tuberculosis patient. It has become increasingly clear that there is no "magic bullet," and that an accurate serological test for active TB will require a panel of antigens and a multiplexed approach. In an effort to identify a collection of antigens from which to develop a multiple antigen serological test, we developed a full proteome microarray made up of approximately 4000 different M. tuberculosis proteins. The array was probed with 257 culture-confirmed TB cases and 307 non-TB controls from 9 different clinics in Africa, Asia, South America and Canada. Background reactivity against Mycobacterium was noted in all non-TB subjects with the highest in Africa followed by Asia, South America and Canada. Differentially reactive antigens were identified that are significantly more reactive in the TB cases than controls. Smear positive cases are more reactive to these antigens than smear negative TB cases. ELISA assays using eight of the antigens together in a multiplex assay could differentiate between TB cases and controls with 91% sensitivity and 83% specificity. These results provide a framework for understanding the humoral immune response to M. tuberculosis infection and for developing more accurate serodiagnostic tests.

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DIAGNOSIS AND MOLECULAR CHARACTERIZATION OF CRYPTOSPORIDIOSIS AND CYCLOSPOROSIS ON INFECTED CHILDREN UNDER FIVE YEARS OLD FROM A NGÖBE-BUGLE COMMUNITY IN WESTERN PANAMA

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The Ngöbe-Bugle is an ethnic group that is located in several rural regions of Western Panama, where the sanitary conditions are frequently very poor. These conditions are favorable for the transmission of several intestinal parasitic infections mainly in children. *Cryptosporidium* sp. and *Cyclospora cayetanensis* are intestinal coccidian that causes diarrhea and malnutrition in children. In Panama, few studies have been conducted to establish the prevalence of intestinal coccidian particularly in Amerindian communities. The aim of this study was to determine the prevalence and genetic characteristics of *Cryptosporidium* spp. and *C. cayetanensis* in 236 children under five years old, from two Ngöbe- Bugle communities. Stool samples were concentrated by acetate ethyl formol technique and the sediment stained with Kinyoun´s stain. *Cryptosporidium* infections were confirmed/characterized by a PCR-RFLP analysis using the SSU rRNA gene as a molecular marker. Further genetic diversity of *Cryptosporidium hominis* positives samples were assessed by sequence analysis of the GP 60 gene. The results revealed that 75/236 (31.7%) evaluated samples were positive for *Ascaris lumbricoides*, 64/236 (27%) for *Giardia lamblia*, 22/236 (9.3%) for hookworms, 11/236 (4.7%), for *Trichuris trichiura,* 15/236 (6.3%) for *Entamoeba histolytica/* E. dispar complex and 97/236 (41%) for non pathogenic intestinal protozoa. The frequency found for intestinal coccidian was of 4.6% (11/236), 2.5% (6 /236) for *Cryptosporidium* spp, 1.3% (3/236) for *Cryptosporidium hominis,* 0.8% (2/236) and 2.2% (5/236) for *C. cayetanensis.* In addition *C. hominis* subtype Ie was confirmed in one sample by the sequencing analysis of the GP 60 gene. This study demonstrates a high prevalence of intestinal

parasites, including coccidian, in the evaluated Ngöbe- Bugle children. Furthers studies are necessary to establish the role of these parasites in the general health status of this ethnic group.

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CYCLOSPORA CAYETANENSIS IN A PEDIATRIC HOSPITAL IN MORELIA, MÉXICO

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Cyclospora cayetanensis affects immunocompetent and immunocompromised individuals and has been associated with food and waterborne gastrointestinal illness characterized by watery and persistent diarrhea and abdominal pain. Cyclosporiasis has been associated with traveler's diarrhea and the consumption of fresh fruits and vegetables. In the present study, stool samples from 8,877 children were examined for ova and parasites at the Pediatric Hospital of Morelia from 2000 to 2009. Sixty children (0.67%) had *Cyclospora* in their stools. Diarrhea (33.3%), abdominal pain (31.6%), and vomiting (15%) were the most frequent symptoms. Most of the cases (93.3%) were observed between June and August, the rainy season. In 45 children, *Cyclospora* was the only parasitic pathogen detected, while 8 children (13%) also had commensal parasites with an overall co-infection in 15 (25%) children. Our findings suggest that *C. cayetanensis*, a parasite with unique geographical distribution, may be endemic in Michoacán, showing a characteristically seasonal pattern.

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EVOLUTION OF SEROLOGICAL TESTS OF TOXOPLASMOSIS IN PREGNANT WOMEN FROM SENEGAL

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Toxoplasmosis is a well-known disease in Europe where its epidemiology was well studied in many countries. In many African countries including Senegal, toxoplasmosis is not subject of a real understanding. The objective of this study is to reassess the toxoplasmosis antibodies prevalence among pregnant women during pregnancy medical surveillance. The test has been performed in 941 pregnant women at the laboratory of parasitology and mycology at Le Dantec teaching hospital from 2002 to 2006. immunoenzymatic method in solid phase has been used. To accomplish this evaluation, two serological tests (S1 and S2), using venous blood at 3 weeks of interval, are carried out among these pregnant women. The second serology will allow confirming a toxoplasmosis from a immune response, or a non specific antibody fixation. From the 941 patients tested, we found a prevalence of 7,7% and 0% for (IgM+IgG-) respectively at serology S1 and S2; 23,3% and 24,3% for (IgM-IgG+), 11,3% and 10,2% for (IgM+IgG+). 34,5% of pregnant women present toxoplasmosis antibody. These data confirm the presence of toxoplasmosis among pregnant women in Dakar

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TOXOPLASMOSIS HOSPITALIZATION TRENDS IN THE UNITED STATES, 1993-2008

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Trends for toxoplasmosis-related hospitalizations have not been reported recently in the United States. Toxoplasmosis-related hospitalizations often occur in persons with HIV infection and other causes of advanced immunosuppression. Using the National Inpatient Sample (NIS), a component of the Healthcare Cost and Utilization Project, we examined trends in toxoplasmosis-related hospitalizations by HIV infection status from 1993 through 2008. The NIS is designed to represent a 20% sample of U.S. community hospitals and includes information on up to 8 million hospital discharges per year from approximately 1,000 hospitals. The NIS is weighted to produce national estimates. States included in the NIS increased from 17 in 1993 to 40 in 2007. ICD 9 codes 130-130.9 were used for toxoplasmosis and 042-044/795.8/V08 for HIV infection. Estimated HIV-associated toxoplasmosis hospitalizations increased from 9,395 (95% confidence limits [CL] 6,902, 11,889) in 1993 to 10,583 (95% CL 7,628, 13,537) in 1995 then dropped sharply to 3,643 (95% CL 2801, 4485) in 2001 with similar levels thereafter. The rate of HIV-associated toxoplasmosis hospitalizations among all HIV-related hospitalizations decreased from 3.33% (95% CL 3.18%, 3.49%) in 1993 to 1.25% (95% CL 1.15%, 1.35%) in 2008. Non-HIV associated toxoplasmosis hospitalizations remained relatively constant from 1993-2008 in approximately the 400-800 range (associated percents were.0020% [95% CL.0017,.0024] and.0015% [95% CL.0012,.0018], respectively). HIV-associated toxoplasmosis hospitalizations dropped markedly after 1995 when highly active antiretroviral therapy for HIV infection was introduced, however, hospitalizations have decreased relatively little after 2000 suggesting that some HIV-infected persons are being tested late or antiretroviral therapy is failing due to resistance, poor compliance, or other reasons. The number of non-HIV associated toxoplasmosis hospitalizations has remained more stable.

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HIGH *CRYPTOSPORIDIUM PARVUM* ANTI-IGG SEROPREVALANCE AMONG HIV-POSITIVE ADULTS IN LIMPOPO AND OTHER REGIONS OF SOUTH AFRICA

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Cryptosporidium spp. are common causes of persistent diarrhea associated with stunting and likely cognitive impairments in malnourished children, and can be life threatening in severely immunocompromised hosts. Literature remains sparse, however, regarding region-specific exposures, and host mechanisms conferring immunity are unclear. A cross-sectional study was conducted to determine the seroprevalance of *Cryptosporidium parvum* in the Limpopo region in South Africa. Banked frozen plasma from 194 HIV-positive adults (11-69 years of age) in Limpopo and neighboring provinces, and recently collected plasma from 58 University of Venda (UNIVEN) healthy volunteers were screened for *C. parvum* IgG antibody with a crude *C. parvum* antigen (Iowa Strain, Waterborne, Inc.) ELISA. Using a previously defined cut-off value of 1.8 times the internal negative control optical density (OD) (mean OD negative control=0.186±0.05) on each plate, the seroprevalence was 70.6% among HIV-positive patients and 32.8% among UNIVEN students (P<0.001). Seroprevalence was high throughout Limpopo as well as neighboring provinces (50-100%). Anti-*Cryptosporidium* IgG was detected in 29 of 44 (65.9%) patients with advanced HIV disease (AIDS or WHO Stage 4). An age-matched comparison between the two groups showed increased risk for anti-*C. parvum* IgG in those with HIV (OR=2.99; 95% CI: 1.48-6.04). In a parallel pilot assay, among twelve of the healthy UNIVEN students, there was no correlation seen between seropositivity and IFN-γ released following whole blood stimulation (QuantiFERON®-CMV**,** Cellestis) with an identical crude *C. parvum* antigen (R^2=0.032). The sustained *C. parvum* IgG antibody responses seen throughout adulthood imply ongoing exposures in rural regions of South Africa. The discrepant ELISA and IFN-γ release assay results warrant further field investigations and laboratory models to elucidate the host immune response to this ubiquitous pathogen.

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DEVELOPMENT OF A NESTED PCR PROTOCOL BASED ON INTERNAL TRANSCRIBED SPACER (ITS) REGION FOR RAPID DETECTION OF HUMAN-PATHOGENIC *CYCLOSPORA CAYETANENSIS* PARASITES

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Cyclospora cayetanensis is a human-pathogenic coccidian parasite causing acute diarrheal disease. Although it is endemic to tropical countries, it has been reported from several geographic regions worldwide. This parasite has been identified as the cause of several foodborne outbreaks in the United States and Canada associated with imported produce, predominantly raspberries. Multilocus genetic characterizations have proven to be advantageous for tracing diminutive genetic variations in several human-pathogenic organisms with low natural genetic diversity including apicomplexan parasites. In our previous study we have examined the 70 kDa heat shock protein (HSP70) gene *C. cayetanensis.* In this study we have described the development of a nested PCR protocol based on the internal transcribed spacer (ITS) region for rapid detection of *C. cayetanensis* parasites in humans. This newly developed nested protocol was tested and authenticated by PCR amplification and nucleotide sequencing. Eighteen human *C. cayetanensis* isolates from three endemic regions including Nepal, Mexico, and Peru were PCR amplified using this ITS primer set. Analysis of the generated ITS nucleotide sequences revealed the *C. cayetanensis* parasites to be a genetically distinct species within the genus *Cyclospora*. This newly developed ITS-based nested PCR protocol provides another useful genetic marker for rapid detection of *C. cayetanensis* parasite in future.

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SEROPREVALENCE OF ANTIBODIES TO *TOXOPLASMA GONDII* IN MALI

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The protozoan parasite *Toxoplasma gondii* is widely distributed throughout the world and its prevalence varies considerably by countries based on local behavioral and environmental risk factors. Toxoplasmosis infection is normally subclinical in immunocompetent adolescents and adults, but *T. gondii* is a prominent opportunistic pathogen associated with AIDS. Congenital infection can have severe consequences on fetus and infants. We conducted a serological survey on existing 650 serum samples collected for malaria studies to assess the seroprevalence of toxoplasmosis in an urban and a rural setting of Mali. Antibody levels were measured using a modified agglutination test assay. A seroprevalence of 24.7% and 26.8% % was observed in adults from the urban (Bamako) and rural setting (Kolle) respectively. No significant difference was observed between the seroprevalence in men *vs.* women. In the rural village of Kolle, seroprevalence rose from 0% in infants (< 1 year) to 0.8% (1-5 yr), 2.7% (6-10 yr), 11.3% (11-15 yr), and 26.8% (>15). The seroprevalence was significantly different between children <10 and the 11-15 yr age group ($p < 10^{-3}$), and between 11-15 and adults ($p = 0.04$). IgG Serum titers in the population increased in parallel with seroprevalence. Modeling the observed age distribution suggests a seroconversion rate of ~2%/yr. This study suggests that congenital toxoplasmosis may be an under-studied public health concern in Mali.

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CLONORCHIASIS: AN EMERGING AND UNDERESTIMATED FOODBORNE TREMATODIASIS IN CHINA

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Clonorchiasis, caused by the oriental liverfluke, *Clonorchis sinensis,* is a major foodborne trematodiasis endemic to parts of Asia including China, Korea, Taiwan, Vietnam, and a small part of Russia neighboring north-eastern China. A conservative estimate suggests that at least 35 million people are infected by this parasite in these regions. In China, despite archaeological evidence suggesting that human infections could be traced back to at least 2,300 years ago, distribution of endemic areas and population affected by the disease was unclear until the first national survey in 1990, indicating that infected people were distributed in 22 provinces/cities with an overall prevalence of infection 0.31% and highest infection (1.82%) in Guangdong Province. In the second national survey during 2002-2004, however, anoverall 75% increase in human prevalence of infections was observed compared to the first national survey, with particularly significant increases in Guangdong, Guangxi, and Jilin where 182%, 164%, and 630% increases were seen, respectively.In Guangdong Province, the recent survey indicated that there are 63 endemic counties where human infections are reported to range between 0.2% and 50.3% at the county/city level. Over 5 million people are estimated to be infected in Guangdong. Although the survey indicated that consuming raw or undercooked fish infected by the parasite and poor sanitation causing fish pond contamination are the main risk factors, little is known about the public health impact (e.g. disease burden) in these endemic areas, transmission pathways beyond the risk factors, and ecology of the parasite and its intermediate hosts. Research needs and challenges in controlling clonorchiasis in China are discussed.

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EVALUATION OF THE EFFICACY OF OXFENDAZOLE AGAINST *FASCIOLA HEPATICA*

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Fasciola hepatica is the most important liver parasitic disease of ruminants in wide areas of the world, with economic implications due to reduced milk production, meat or wool. Fasciolosis is also a zoonotic disease causing considerable morbidity by damages in the human liver and biliary system. Although numerous treatments have been used in the past on humans and animals, most were poorly effective and now the drug of choice is triclabendazole. Triclabendazole resistance has already been demonstrated in small ruminants in different countries. A potential single dose alternative treatment, oxfendazole, has not yet been evaluated for fasciolasis. In this study, we evaluated the efficacy of oxfendazole in 40 adult sheep naturally infected with *F. hepatica*. Sheep belonged to a community in an endemic area to fasciolasis. All the animals were screened for *Fasciola* eggs one day before the beginning of the study (day 0) by sedimentation method to confirm that their fecal egg counts was higher than 2 eggs per gram (*epg*). The animals were randomly allocated in two groups of 20 sheep each. The first group was left untreated (control), while the second group (treatment) was treated orally with oxfendazole at a single dose of 30 mg/kg of body weight. Fecal samples were taken from each animal 10 days after the treatment, and the number of *Fasciola* eggs was determined with the same method. In the day 0, all animals (two groups) had on average 4.65 (2-28) *epg.* Ten days after

treatment, the control group had 7.5 (2-37) *epg*, while and the treatment group had 0 *epg*. A single dose of 30 mg/k of oxfendazole is safe and 100% efficacious in sheep infected with *F. hepatica*.

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FIVE SPECIES OF ECHINOSTOMES RECOVERED FROM HUMANS IN KHAMMOUANE PROVINCE, LAO PDR

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Echinostomes (= family Echinostomatidae) are intestinal trematodes of humans and animals, and can cause severe epigastric or abdominal pain accompanied by diarrhea, easy fatigue, malnutrition, and rarely mortality in human infections. In the present study, 5 species of echinostome flukes were recovered after praziquantel treatment of 9 people living along the Mekong River in Lao Peoples' Democratic Republic (Lao PDR). The surveyed areas were riverside (tributaries of the Mekong River) villages in Khammouane Province. A total of 1,242 fecal samples were collected and examined using the Kato-Katz thick smear technique. Echinostome eggs, species undetermined, were detected in 9 people (0.67%), i.e., 6 male and 3 female patients. These egg positive people were given a single oral dose of 10-20 mg/kg praziquantel and purged with 20-30 g $MgSO_4$. Worms were collected from their diarrheic stools, fixed in 10% formalin, stained with acetocarmine, and morphologically identified. A total of 52 echinostome specimens were recovered. They consisted of 5 species, including 6 specimens of *Echinostoma revolutum*, 5 of *Echinostoma macrorchis*, 4 of *Euparyphium murinum*, 2 of *Artyfechinostomum malayanum*, and 31 of *Echinochasmus japonicus*. All these echinosotme species are reported for the first time in Lao PDR. *E. murinum* turned out to be a new zoonotic parasite so far as the world literature are concerned. We report echinostomiasis as one of the endemic trematode infections among villagers of Khammouane Province, Lao PDR.

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SEROLOGICAL DIAGNOSIS OF NORTH AMERICAN PARAGONIMIASIS BY WESTERN BLOT WITH *PARAGONIMUS KELLICOTTI* ADULT WORM ANTIGEN

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Human paragonimiasis is an emerging disease in Missouri. 12 cases have been diagnosed since 2006, and many patients presented with serious illnesses. The infection is caused by *Paragominus kellicotti* (Pk), which is highly prevalent in crayfish in Missouri rivers and streams. The purpose of this study was to develop and evaluate an antibody serology test for diagnosis of Pk infections. We infected gerbils with Pk metacercariae and recovered adult parasites 6 wks later. An adult worm antigen extract was used to detect IgG antibodies by Western blot on 4-12% gradient gels. The test was evaluated with sera from 30 healthy Americans (HA), 39 sera from patients with other helminth infections (OHI, *Strongyloides, Schistosoma, Fasciola, Echinococcus*), 7 sera from patients infected with *P. westermani* (Pw, Philippines), and 10 from patients with proven *P. kellicotti* infection (Pk, confirmed by CDC Western blot using *P. westermani* antigen or by recovery of eggs from sputum/BAL). Two other sera were tested from 2 suspected Pk (sPk) cases that were negative by Western blot at CDC. All 10 Pk sera and both sPk sera contained antibodies to an antigen at 36 kDa and a doublet at 24/26 kDa. Six of the 10 PK sera also recognized an antigen at 8 kDA. Some sera also labeled other antigens at 4, 12, 14, 44, 47, 49, 55 and 62 kDa. All 7 Pw sera labeled the 36kDa antigen, but only 2 labeled the 24/26 kDa antigens, and 6 labeled the 8 kDa antigen. Several HA and OHI sera weakly labeled antigens at 14, 25, 29, 44, 47, 49, 55 and 62 kDA, but none labeled antigens at 36, 24/26, or 8Kda. Based on these results, we consider sera that label either the 36 kDa or the 24/26 kDA bands to be positive for antibodies to *P. kellicotti*. Antibody responses were significantly reduced from baseline in sera from two patients that were collected 6 months after praziquantel therapy. Thus, the Pk Western blot appears to be highly sensitive and specific for diagnosis of paragonimiasis, and it may also be useful as a test of cure. Pk antigen appears to be superior to Pw antigen for diagnosing Pk infections.

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SYNERGISTIC REVERSAL OF PATHOLOGY DUE TO CHRONIC *SCHISTOSOMIASIS MANSONI* BY PRAZIQUANTEL AND DT_{390}-IL-18 TARGETED PLASMID IMMUNOTOXIN IN EXPERIMENTAL BALB/C MICE

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Patient with chronic schistosomiasis often fail to resolve hepatic fibrosis after antehelminthic treatment. DT_{390}-IL18 immunotoxin suppresses immunopathology in schistosomiasis by destroying antigen activated APCs and T cells. Therefore, we studied the synergistic effects of DT_{390}-IL-18 and Praziquantel (PZQ) on the resolution of pathology due to chronic *Schistosoma mansoni* infection in mice. 13 weeks after exposure to 25 *S. mansoni* cercariae, mice were treated with Placebo, PZQ, DT_{390}-IL-18, or PZQ+DT_{390}-IL-18. Pathology was assessed by granuloma size, hepatic weight, hepatic proline, hydroxyproline, collagen I and III and fibronectin content and infiltrating cells phenotypes. Mice that received PZQ+DT_{390}-IL-18 had a very significant increase in the rate of resolution of hepatic fibrosis (+65%) when compared to placebo treated control, PZQ (+35%) or DT_{390}-IL-18 (+23%) alone. Similar synergistic suppression of $CD4^+$, IL-18 αR, Th1 and Th2 cells in hepatic granulomas and the induction of apoptosis were also observed. Therefore, the resolution of immunopathology in mice with chronic schistosomiasis is enhanced with a combination of chemotherapy and immunotherapy. This therapeutic approach could be of potential use in humans demonstrating pathology due to chronic hepatosplenic schistosomiasis.

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CPG-ODN REPRESENTS AN ALTERNATIVE ADJUVANT TO BE USED IN A VACCINE FORMULATION AGAINST SCHISTOSOMIASIS

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Currently schistosomiasis control is mainly based on chemotherapy, but in spite of decades of mass treatment, the number of infected people remains constant. A vaccine that induces even a partial reduction in worm burdens could reduce pathology and limit parasite transmission. The surface of the *Schistosoma mansoni* schistosomula (Smteg) is an important target for host immune system attack since it represents the interface between host and parasite and thus is a potential candidate

for vaccine development, recently we have shown that Smteg is able to activate dendritic cells *in vitro* and also can induce 43-48% protection in mice when in association with Freund`s adjuvant. In this study we evaluated the ability of different adjuvants (alum or alum +CpG) in association with Smteg to induce protection in a three dose immunization protocol in mice. Thirty days after the third dose, mice were infected and 50 days pos-infection mice were perfused. During the immunization, blood samples were collected for the ELISA assay. A stool examination was also performed. The number of eggs in the liver and intestine wall was determined. The profile of the immune response induced by each formulation was determined by cytokine measurement and immune cells characterization. In the group of mice immunized with Smteg alone or with alum, no protection was observed, however immunization with Smteg + alum+ CpG-ODN were able to induce a 43-51% reduction on adult worm burden; 35 % in the number of eggs/g in the liver and intestine; 54% in the number of eggs/gram of faeces. The protective immunity observed in Smteg/alum/CpG-ODN group was associated with a increase production of specific IgG2c antibodies, significant production of IFN-γ and IL-10 by CD4+ cells, activation of CD4+ cells, and increased expression of CD86 in F4/80+ cells. These results not only confirm Smteg antigens as potential candidates to be used in a vaccine formulation but also indicate an alternative adjuvant and the immune response associated with protection.

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HISTONE MODIFYING ENZYMES (HMES) OF *SCHISTOSOMA MANSONI* AND *S. JAPONICUM*

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Schistosomes infect over 200 million individuals in more than 75 countries in tropical or subtropical regions and cause more than 280,000 deaths annually. The genomes and predicted proteome sequences of *Schistosoma mansoni* and *S. japonicum* were recently published providing an opportunity to identify new drug candidates. Histone modifying enzymes (HMEs) are major players in the regulation of chromatin epigenetic modifications. Furthermore, aberrant epigenetic states are often associated with cancer, leading to great interest in HMEs as therapeutic targets. In order to choose potential drug targets for further study, we have characterized all enzymes involved in either acetylation or methylation histone modifications: histone deacetylases (HDAC), histone acetyltransferases (HAT), histone methyltransferases (HMT), and histone demethylases (HDM). We analyzed the *S. mansoni* and *S. japonicum* predicted proteomes to identify and classify these HME families through computational approaches. Functional annotation was performed mainly to yield insights into the enzymes involved in epigenetic modifications, which could be relevant to its development. By using Hidden Markov Models, we have identified a total of 54 and 39 HME proteins in the predicted proteomes of *S. mansoni* and *S. japonicum*, respectively. The results show that *S. mansoni* and *S. japonicum* code for proteins in all the selected HMEs families, with the largest number of proteins found in the HMT subfamilies. Individual annotations of *S. mansoni* proteins revealed 14 splicing variants as well as some incorrect predictions identified in both species. Only six HMEs had been experimentally studied and the others were previously annotated only by automatic sequence similarity-based methods as described elsewhere. Thus, we have improved the annotation of 23% of *S. mansoni* HMEs. As we continue this work, we will validate some HMEs as molecular targets using RNA interference to silence the corresponding genes in schistosomula and analyze the resulting knockdown by quantitative PCR and potential phenotypes.

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SCHISTOSOMA MANSONI TEGUMENT (SMTEG) MODULATES THE EXPERIMENTAL ALLERGIC ASTHMA

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Allergic inflammations are directed by Th2 cells activation that produces large amounts of IL-4, IL-5 and IL-13. These inflammatory mediators induce IgE production and eosinophilia. The schistosomula is the first stage to keep contact with the host immune system activating the antigen presenting cells and promoting the lymphocytes B and T differentiation. Although immune responses between helminthes infections and asthma are similar some studies performed in endemic areas of *Schistosoma mansoni* showed low prevalence of allergic diseases. This modulation has been associated to interleukin (IL)-10 production and an increased number of T regulatory cells. Many factors may be acting to inhibit the allergic asthma induction in mice associated to schistosomiasis, which involve both innate immune responses as adaptive. Our goal is to investigate the mechanisms responsible for the immune modulation of asthmatic response after the Smteg (*S. mansoni* tegument preparation) intraperitoneal injection. Balb/C mice were divided in three groups (n=5) PBS, Asthma and Smteg/Asthma. All groups were immunized twice with 10µg of ovalbumin chicken egg (OVA) plus alum subcutaneously in a 15 days interval. One week after the first one, mice from Smteg/Asthma group were immunized intraperitoneally with 25 µg of Smteg. The ASTHMA groups were challenged by OVA aerosol to develop asthma, one week after the second immunization and after twenty four hours mice were euthanized. Bronco-alveolar lavage (BAL) was performed to eosinophils counting and the lungs were collected for cytokines (IFNγ, IL-4, IL-10, IL-17, IL-13) and chemokines (CCL2, CCL3, CCL5, CCL11) analysis. The number of eosinophils was higher in ASTHMA group compared to PBS. SMTEG/ASTHMA presented reduced levels in CCL11, IL-17, IL-13 and eosinophils numbers and high levels in interleukin 10 (IL-10) when compared to ASTHMA group. IL-4, CCL2 and CCL3 did not show statistical difference between these groups. Treatment with Smteg modulated the number of eosinophils with increase in IL-10 in allergic asthma.

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VACCINE EFFICACY OF THE *SCHISTOSOMA JAPONICUM* INSULIN RECEPTORS

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Schistosomiasis, which affects 200 million people worldwide and is responsible for hundreds of thousands of deaths annually, continues to be a significant public health problem. We have identified two types of insulin receptors from the blood fluke, *Schistosoma japonicum*, SjIR-1 and SjIR-2. SjIR-1 is located on the tegument basal membrane and the internal epithelium of adult worms, whereas SjIR-2 is located in the parenchyma of males and the vitelline tissue of females. The incubation of adult worms *in vitro* with HNMPA, which inhibits autophosphorylation of the HIR that is involved in the regulation of glucose uptake in mammalian cells, and anti-SjIRs antibodies respectively resulted in a significant decrease in worm glucose and glycogen levels, suggesting the important role of SjIRs in regulating glucose uptake, similarly to that described for mammalian cells. Vaccination of mice with recombinant SjIRs followed by cercarial challenge infection with *S. japonicum* resulted in statistically significant the stunting of adult worms ranging from 22-25% in the SjIR-1 vaccinated group to 37-42% in the SjIR-2 vaccinated groups, highly significant reductions in faecal eggs in both the SjIR-1 (66%) and SjIR-2 (68%) vaccinated groups, although there was no significant reduction

in adult worm burdens. Vaccination also resulted in a reduction in liver egg numbers in the SjIR-1 (33%) and SjIR-2 (5.4%) vaccinated groups. Based on repeated vaccination trials, the resulting reductions in worm length, liver and intestine egg numbers and faecal eggs all indicate a significant depression of parasite growth and a subsequent reduction in parasite fecundity. The highly significant decreases in faecal egg output is noteworthy and suggests an application as a veterinary vaccine which could prevent transmission of zoonotic schistosomiasis to the human endemic population. Further development and validation of the vaccine is currently underway. This work also provides important new information on the role of SjIRs in the biology of *S. japonicum*, and may suggest their novel use as vaccine targets against schistosomiasis and other debilitating parasitic diseases.

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TEMPO-SPATIAL DISTRIBUTION OF *SCHISTOSOMA JAPONICUM* IN THE YANGTZE RIVER

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Schistosomiasis is caused by contact with schistosome-infested water when washing or bathing. The flooding period between May and September each year of the Yangtze River, is the high-risk season of infection of *Schistosoma japonicum*, and the resultant high re-infections. The present study was to investigate the tempo-spatial distribution pattern of *S. japonicum* cercariae in waterbody of Jiangsu section of the Yangtze River. The water infectivity of *S. japonicum* was determined along the the Yangtze River from May to September by using sentinel mice, and the dynamic database of water infectivity was established. Among the 4 500 sentinel mice which were placed in 45 sites, 4 411 were recovered, with a recovery rate of 98.33%. A total of 4 370 mice were dissected and 23 infected, with a total infection rate of 0.53%, and the infection rates of *S. japonicum* in mice were 0.23%, 0.23%, 0, 0.45% and 1.73%, respectively month by month. Fifty-five adult worms were collected, with mean worm burden of 2.39 worms per mouse in infected sentinel mice, and the mean worm burdens of the infected sentinel mice were 1.00, 2.00, 0, 1.50 and 2.87 worms per mouse, respectively month by month. From May to September, 12 sites with infected sentinel mice were found, accounting for 24.44% of the total forecast and surveillance sites, and number of sites with infected sentinel mice were 1, 2, 0, 1 and 8, respectively, with occurrence rates of sites with infected mice of 2.22%, 4.44%, 0, 2.22% and 17.78%, respectively, and the constituent ratios of the sites with infected mice were 8.33%, 16.67%, 0, 8.33% and 66.67%, respectively month by month. The occurrence rate of sites with infected mice in September was significantly higher than that in June (χ^2=4.05, *P*=0.044). And the top infection rates of *S. japonicum* in sentinel mice were found in sluices, being 2.08%-6.45%. It is concluded that the infection of *S. japonicum* during the period of flood season in the Yangtze River exhibits bimodal distribution. Top water infectivity appears in September, July is the period of metagenesis of infected snails in marshland areas, and the critical time for prevention and control of acute schistosomiasis is between August and October.

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PREVALENCE OF URINARY SCHISTOSOMIASIS IN OGUN STATE, SOUTHWEST NIGERIA

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This study, initiated by the Federal Ministry of Health Abuja, Nigeria, is aimed at providing prevalence distribution data of urinary schistosomiasis in Ogun State, Nigeria, as part of an ongoing effort in planning a national control program of the disease by preventive chemotherapy. In October 2009, a cross-sectional prevalence survey of *Schistosoma haematobium* infection among school children aged 9-11 years was carried out in 15 of the 20 local government areas (LGA) of Ogun State, Nigeria. One study community was selected in each LGA. Following informed consent from the parents and community leaders, urine samples were collected in clean specimen bottles from 50 randomly selected pupils from each of the 15 study communities. The geographical coordinates of the study communities were recorded. The urine samples were examined visually for macrohaematuria, tested with reagent strips for microhaematuria and examined microscopically for *S. heamatobium* eggs. Positive diagnosis was based on detection of macrohaematuria, microheamaturia and / or *S. haematobium* eggs in urine. Out of 735 pupils (367 males and 368 females) examined, 194 (26.4%) were positive for *S. heamatobium* infection. The infected children were found in 13 of the 15 LGAs visited. The 194 infected, represent a prevalence of 30.4% of the 638 children examined in the 13 endemic LGAs. Prevalence in the endemic communities varied from the lowest of 2% at Idode in Ijebu North LGA to the highest of 84% at Imala Odo in Abeokuta North LGA. Prevalence was above 20% in seven LGAs, that is, Odeda, Abeokuta North, Yewa South, Yewa North, Obafemi Owode, Ewekoro and Ijebu East. Prevalence was highest (≥ 40%)among the fishermen communities situated on the banks of or close to water reservoirs in water development project areas of the state. Prevalences of infection among the two sexes were comparable with 27.8 % in the males and 25.0% in the females (p>0.05)

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CHARACTERIZATION OF THE NOVEL SCHISTOSOMAL RECEPTOR, SMGPR-3

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The main causative agent of schistosomiasis, a disease which infects over 200 million people worldwide, is the parasite *Schistosoma mansoni*. Treatment of the disease is primarily with praziquantel (PZQ). There is an increasing fear that with the widespread use of the drug, and the lack of an available alternative, PZQ will lose its effectiveness. We are currently researching the schistosome nervous system to gain insight into this area. The nervous system coordinates many vital functions in the worm and is considered to be an excellent target for anti-schistosomal drugs. Recently, we have discovered a new group of schistosomal biogenic amine (BA) G-Protein Coupled Receptors (GPCRs), the smGPRs, which likely have a neuronal function. SmGPR-1, -2 and -3 have been cloned, and their pharmacological profiles determined by our lab. These receptors differ from the BA GPCRs of the human host in both sequence and function. SmGPR-3 was immunolocalized in the adult worm and shown to be expressed abundantly in the central and peripheral nervous system, including peripheral neurons which innervate the worm musculature, indicating a neuromuscular role. To further characterize smGPR-3, a predicted binding site (D3.32) was mutated and functional expression studies were performed, to assess receptor activity. The wild-type and mutant plasmid constructs were individually expressed in yeast cells which, upon successful interaction with ligand and receptor activation, express a reporter gene, allowing for the cells to be selectively grown in media. Cell growth is then quantitatively assayed, using the Alamar Blue fluorescence assay, as a measure of receptor activation. The mutants showed varied levels of responsiveness to ligand, indicating the site's importance. RNAi knock-down of smGPR-1, -2, and -3 in schistosomulae, followed by video analysis was performed. Targeting of smGPR-3 results in a decrease in worm motility by approximately half, as compared to the control, indicating a role in motility for the receptor.

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SUPPRESSION OF IMMUNOPATHOLOGY IN MURINE *SCHISTOSOMIASIS MANSONI* BY A TARGETED PLASMID CONTAINING IMMUNOTOXIN, DT_{390}-IL-18-SRA GENE (STUDIES OF *IN VITRO* AND *IN VIVO* EFFICACY)

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This study was undertaken to evaluate both *in vitro* and *in vivo* effects of DT_{390}-IL-18-SRα immunotoxoin on the development of immunopathology in murine schistosomiasis mansoni. The antiproliferative effect and *in vitro* granuloma formation (IVGF) inhibition were evaluated using methyl thaiazolyl tetrazolium and IVGF index respectively. *In vivo* evaluation, mice were divided into four groups of twelve mice each: Group1 normal non infected (-ve control), Group 2, 3 and 4 were infected percutaneously with 25 cercariae. After 6 weeks post infection, Group2 was treated with PBS (+ve control), Group3 was treated with plasmid DNA (50 µg) embedded with cationic liposome in 50 µl PBS, injected intramuscular in the hind limbs once daily for two weeks and Group4 was treated with DT_{390}-SRα (mutant control). After animals sacrification, liver was removed for histopathological studies. DT_{390}-IL-18-SRα showed suppression of spleen lymphocytes proliferation and IVGF in a dose dependant manner as well as suppression of liver granulomas size by 80%, while mutant toxin had no significant suppression (P › 0.05). In conclusion, the immunotoxin showed selective toxicity to antigen activated lymphocytes *in vitro* with reduced clinical and pathological severities of the disease.

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ACETYLCHOLINE-GATED CHLORIDE CHANNEL SUBUNITS AS MODULATORS OF *SCHISTOSOMA MANSONI* MOTOR FUNCTION

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Currently, praziquantel is the only treatment available against parasitic trematodes of the genus *Schistosoma*. Thus, discovery of new drug targets against schistosomes is of the utmost importance. Historically, drugs targeting modulators of worm motility, particularly the cholinergic system, have been effective against helminth parasites. Previous studies have shown that acetylcholine and other cholinergic agonists have strong paralytic effects on schistosomes *in vitro*. Paralysis is mediated by nicotinic acetylcholine receptors (nAChRs) and is associated with relaxation of body wall muscles, suggesting an inhibitory neuromuscular effect. Analysis of the *S. mansoni* genome database has revealed that in addition to the expected, excitatory cation-selective nAChRs, there are several nAChRs predicted to form putative anion-selective channels. As cation channels are not known to cause muscle relaxation, we hypothesize that the putative anion nAChRs are responsible for the inhibitory effects of acetylcholine on schistosome movement and are promising drug targets due to their low homology to other nAChRs. The goal of this study is to characterize the putative anion-selective nAChR subunits of *S. mansoni*. Here, we present the results of a bioinformatics analysis of schistosome cholinergic receptors and an RNAi study assessing the role of putative anion-selective nAChRs in *S. mansoni* motor function. SiRNA against 5 nAChR subunits were transfected into Day 0 schistosomulae. Motor phenotypes were then quantified using motion-tracking software both at baseline levels and after treatment with exogenous acetylcholine. We observed significant increases in the frequency of body wall contractions and overall body length in the siRNA-treated samples compared to the negative control. Treatment with exogenous ACh caused no change in phenotype of the siRNA-treated samples but did lead to increases in length in the controls. These results suggest that ACh may act through these putative anion-selective nAChRs as an inhibitory neurotransmitter affecting worm motility.

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EXPRESSION AND ANALYSIS OF *SCHISTOSOMA MANSONI* IMMUNODIAGNOSTIC ANTIGEN, SM29

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The helminthic parasite *Schistosoma mansoni* is one of the causative agents of the neglected tropical disease schistosomiasis, which affects more than 200 million people worldwide. Since 1985, several of the more reliable methods for immunodiagnosis of this disease, FAST ELISA and EITB, have relied on a mixture of proteins from the microsomal fraction of adult worms. The *S. mansoni* microsomal protein preparation contains two species-specific antigens known as Sm29 and Sm25, based on their migration by gel electrophoresis. These proteins have demonstrated excellent sensitivity and specificity by Western blot; however, they have proven to be both expensive and difficult to obtain. The release of the *S. mansoni* genome provides an opportunity to utilize a proteomics approach for the identification of these diagnostic markers and the potential to develop a less expensive, more abundant source of antigen as recombinant protein. For identification of Sm29 the microsomal antigen fraction was analyzed by two-dimensional gel electrophoresis revealing 15 distinct protein spots at the correct molecular weight, 5 of which were immunoreactive by Western blot analysis. Spots corresponding to the Western blot positive proteins were excised from the gel, digested with trypsin and analyzed by mass spectrometry, which revealed each protein came from the same gene product. The gene has been cloned and recombinant Sm29 protein was expressed in a baculovirus expression system, and subsequently tested as an immunodiagnostic marker for *S. mansoni* infection.

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SMTOR IS A NEW CANDIDATE VACCINE FOR SCHISTOSOMIASIS

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Praziquantel is the only chemotherapeutic available for treatment of infection with *Schistosoma* spp., but it does not protect from re-infection. Therefore, there is a need to develop an effective vaccine against schistosomiasis. Vaccine candidates include proteins located on the parasite tegument such as SmTSP-2, Sm29 and SmStoLP-2 and the cytosolic fatty acid binding protein Sm14, but only the last-mentioned is in the clinical trial phase. Here we explored another surface-exposed vaccine target. SmTOR is a tetraspanning orphan receptor expressed highly in *S. mansoni* cercariae. Its extracellular domain 1 (ed1) contains a complement C2 binding sequence that had been described for the *S. haematobium* receptor homologue (ShTOR) and been shown to interfere with complement C2 cleavage *in vitro*. We recombinantly over-expressed SmTORed1 in E. coli, tested its capacity to bind purified C2 and furthermore the occurrence of specific anti-rSmTORed1 antibodies in *S. mansoni* infected and uninfected individuals. To ensure antibody specificity, we performed a competitive ELISA by pre-incubating the positive sera with recombinant SmTORed1 produced as HaloTag fusion protein coupled to a solid support. Lastly, rSmTORed1 was tested as vaccine candidate in a murine infection and challenge model. Purified rSmTORed1 bound complement C2 alike the peptide motif found in ShTOR. We detected specific antibodies against rSmTORed1 in 2/20 (10 %) patient sera and 2/40 (5 %) sera of uninfected individuals. The low occurrence of antibodies in patient sera is possibly due to SmTOR is not being recognized during infection. That uninfected individuals have such antibodies might be due to infection with bird schistosomes (Trichobilharzia). Balb/c mice immunised with rSmTORed1 with CFA/IFA generated significantly high antibody titres that were protective against infection as shown by a significant decrease in worm burden in immunised

versus control animals (60 % reduction). In conclusion, we found that rSmTORed1 is a promising new vaccine target against *S. mansoni* infection.

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A VASA GENE FROM *SCHISTOSOMA MANSONI* FOR DEVELOPMENT AS A MARKER FOR GERMLINE TRANSGENESIS

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Although retrovirus mediated somatic transgenesis in *Schistosoma mansoni* has been reported previously, an important goal is the development of germline transgenesis. Progress towards this might be monitored if germ cells could be identified in cultured schistosomes. Vasa, an ATP-dependent DEAD-box RNA helicase, has germline-specific expression. A reporter transgene driven by a schistosome vasa-like promoter might reveal whether transgenes had reached the germ cells. The present study addressed the identification of a vasa-like gene in *S. mansoni* and characterization of putative promoters of schistosome vasa to drive expression of the firefly luciferase reporter gene. Our findings indicate that the *S. mansoni* genome encodes three putative vasa orthologues identified by BLAST searches and related bioinformatics tools. Phylogenetic relationships were inferred using PHYLIP. The vasa-like orthologues were termed *S. mansoni* vasa-like gene 1 (Smvlg1), Smvlg2, and Smvlg3. A 1.5 kb and 2.0 kb genomic fragment of the Smvlg1 promoter was cloned into pGL3 and was employed to transfect the HT1080 human fibrosarcoma and the HeLa cervical cancer cell lines. Lysates of transfected cells were analyzed for luciferase activity. Also, schistosome eggs were transformed with the Smvlg1 promoter constructs. Total RNA isolated from miracidia hatched from transfected eggs was retrotranscribed into cDNA and luciferase reporter transgene expression quantified by real time PCR. These findings indicated that the 1.5 kb and the 2.0 kb promoter fragments of Smvlg1 were capable of driving reporter gene expression in mammalian cancer cell lines and that the 2.0 kb promoter fragment capable of driving reporter gene expression more efficiently than the 1.5 kb fragment in *S. mansoni* eggs.

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LENTIVIRAL TRANSDUCTION OF THE HUMAN BLOOD FLUKE *SCHISTOSOMA MANSONI*

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We have begun to address whether pseudotyped human immunodeficiency virus type 1 (HIV-1) (a lentivirus) might have utility for transgenesis and other functional genomics in schistosomes. We investigated early steps of lentivirus infection including attachment of virions to the schistosome tegument, reverse transcription to synthesize viral cDNA, and integration of the provirus into the schistosome genome. 293T/17 producer cells were transfected with plasmid encoding wild type HIV-1 isolate NL4-3 and vesicular stomatitis virus-glycoprotein (VSV-G) encoding plasmid to produce lentivirus virions pseudotyped with VSV-G. Schistosomes were incubated with VSVG-HIV virions in the presence of polybrene. At intervals from 0 to 4 hours, schistosomes were washed and the surface proteins cross-linked with formalin. Using a VSV-G specific antibody, time course dependent immunolocalization of VSV-G was observed to both schistosomules and adult worms, with fluorescence signals increasing with time. Downstream events were investigated at days one and two after transduction. Total DNA was extracted from schistosomes and used as template for quantitative real-time PCR measuring products of reverse transcription and integration. One step PCR for reverse transcription products revealed the presence of strong stop and positive strand viral cDNA, and anchored PCR indicated the integration of HIV proviruses in schistosome genome. Findings with control groups exposed to heat inactivated virions and with spinoculation of virions onto the schistosomes provided additional support to the hypothesis that proviral lentiviral transgenes had integrated into schistosome chromosomes. Future studies will address chromosome/ provirus integration junctions, target site preferences and reporter gene activity, and with the aim of establishing VSV-G-pseudotyped HIV-1 as a vector for genetic manipulation of schistosomes.

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SEVERE *SCHISTOSOMIASIS MEKONGI* IN SOUTHERN LAO PEOPLE'S DEMOCRATIC REPUBLIC

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In 2007, within the context of a community-based survey on helminth infections in three villages in Khong District of southern Lao People's Democratic Republic, we identified severe cases of schistosomiasis. We revisited three villages yearly and followed the patients for three years. We identified nine patients with severe schistosomiasis (7 male, 2 female). Mean age of the nine patients was 36 years (range: 5 - 66 years). The leading clinical features were cachexia, hepatosplenomegaly, ascites, splenic varices and rupture of oesophageal varices. Patients were co-infected with *Opisthorchis viverrini* (*n*=6), *Strongyloides stercoralis* (*n*=1) and hookworm (*n*=7). All patients were treated with praziquantel. Three patients improved (case 5, 6, 9), two adult patients (case 2, 3) remained unchanged or the status worsened. Two patients (case 4, 7) died due to oesophageal bleeding. Two new patients were diagnosed in 2009 (case 7, 8). Liver pathology improved after treatment in particular in young patients. Severe chronic schistosomiasis is still present in Laos. Schistosomiasis transmission is currently ongoing as documented by the presence of diseased children. A long-term integrated control intervention including access to treatment, health education, sanitation and infrastructure are urgently required.

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DIHYDROARTEMISININ, A NEW ANTISCHISTOSOMAL AGENT AGAINST *SCHISTOSOMA JAPONICUM*

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Praziquantel is virtually the only current drug of choice for treatment of human schistosomiases. However with the extensive, long-term repeated use of the drug for morbidity control, there is a growing concern that praziquantel resistance or reduced susceptibility may emerge. Screening and development of novel antischistosomal agents, is therefore given high priority. It has been shown that artemisinin derivatives like artemether and artesunate exhibit effectively antischistosomal activities. However, the antischistosomal efficacy of dihydroartemisinin, the main metabolite of the mother compound artemisinins, as well as of the two derivatives, artemether and artesunate, remains unclear. The present study was designed to investigate the *in vivo* activity of dihydroartemisinin against S. *japonicum*. Our finding showed that, single oral doses of dihydroartemisinin (at 300 mg/kg) reduced total worm burdens of 1.07%-64.81% and female worm burdens of 11.90%-90.48%, depending on when, relative to infection, treatment was given, and the greatest reductions was seen when treatment was given either 7 or 35 days post-

infection. However, no marked dose-response relationship was observed. During the schistosomulum stage (7 day), the combined treatment of dihydroartemisinin and praziquantel, or administration of praziquantel, followed by treatment of dihydroartemisinin, both resulted in lower efficacies of dihydroartemisinin against *Schistosoma japonicum*. However, no marked changes of antischistosomal activities were observed when dihydroartemisinin was given first, followed by praziquantel. At adult stage (35 day), a significantly higher antischistosomal efficacy was found for combination therapy with dihydroartemisinin given first, followed by praziquantel, compared to dihydroartemisinin alone, or praziquantel given first followed by dihydroartemisinin. However, no significant difference was observed between the effects of combined treatment of dihydroartemisinin and praziquantel and administration of praziquantel alone. Administration with artemether, artesunate and dihydroartemisinin at multiple doses or in combined treatment damages both juvenile and adult *S. japonicum*, but there were no statistically significant differences among the three drugs at the same dose. It is concluded that, dihyartemisinin is a novel antischistosomal agent against *S. japonicum*.

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TRANSGENE EXPRESSION FROM *MOS-1 MARINER* TRANSPOSON IN *SCHISTOSOMA MANSONI*

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The genome sequence of the *Schistosoma mansoni,* one of the major causative agents of schistosomiasis worldwide, is available in draft format. This genome includes about 12,000 protein encoding genes. The function of most of these remains unknown or poorly understood but, can represent new targets for intervention and control. Insertional mutagenesis of exogenous transposons has been shown to be powerful transgenesis tools as in many species. Previously we have reported that the *piggyBac* transposon is active in schistosome tissues and can integrate into the schistosome genome. The *Mos-1 mariner* transposon, originally isolated from the fruit fly belongs to one of the most widespread transposable element family. As with *piggyBac*, we have been investigating the potential utility of this transposon for functional genomics in schistosomes. Using an excision assay that analyses the donor plasmid backbone, we have also recently determined that *Mos-1 mariner* is transpositionally active in schistosome tissues. In the present study, we have focused on the activity and longevity of the reporter transgene, firefly luciferase, carried as cargo within the inverted terminal repeats of the transposon, using RT-PCR based approaches. Targeting the schistosomule stage of *S. mansoni* at the outset, we investigated the effect of age of the schistosomules on luciferase expression after introduction of the transposon. Second, we investigated the influence of chromosomal integration of the transposon by comparing expression in schistosomules that had been transduced with the donor transposon alone compared with others transduced with the donor plasmid transposon in tandem with mRNA encoding the *Mos 1 mariner* transposase. Third, we monitored the time course of expression and longevity of expression of the luciferase after exposure of the schistosomules to *Mos-1 mariner.* These findings and discussion of the impact of the results will be discussed.

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APPLICATION OF MICROSATELLITE GENOTYPING TO CERCARIAE IN THE INVESTIGATION OF URBAN SCHISTOSOMIASIS

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Schistosomiasis is an endemic disease to parts of Brazil that affects 2-6 million people in 9 states. Caused by *Schistosoma mansoni*, whose intermediate hosts are snails of the species *Biomphalaria glabrata*, being the only transmitter found in the state of Bahia. Historically described as a rural disease, urban transmission of the parasite has been more commonly seen in cities of Brazil. Our goal was to determine the utility of cercariae shed from collected snails in estimating the genetic relationships between urban populations of parasites. Taking advantage of an ongoing malacologic study of all major collections of water in the city of Salvador (total of 158) seven sites were identified as positive for cercariae shedding. Cercarial DNA from 5 sites was extracted and quantified by qPCR. Genotyping assays were performed using 14 microsatellite markers and diversity index used was the Jost's D. Worm and cercariae DNA from laboratory strain maintained at Case Western Reserve University and at Oswaldo Cruz Foundation, respectively, were used as PCR positive control. The total number of alleles observed for all of the markers was 120, ranging from 44 to 91, in the district of Itacaranhas and Pituaçu, respectively. The average effective allele number was similar across all samples. A pairwise comparison of the Jost's D values of all cercarial collections showed a great amount of differentiation and difference between most field collections was as great as that between the positive controls. Only two collections demonstrated potential gene flow between them, the laboratory strain Feira de Santana and Dique do Cabrito (mean Jost D = 0.017). This, however, is spurious since they are reproductively isolated from each other. Therefore, there was no correlation between geographic location and diversity indices. Our results suggest some evidence that snail infections may not reflect the genetic diversity found in the associated human population and that in Salvador, snail examinations may not be useful to assess parasite population structure and dynamics in the human host.

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SPATIAL AND TEMPORAL STABILITY OF *GLOSSINA FUSCIPES FUSCIPES* POPULATIONS IN UGANDA

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Glossina fuscipes, a riverine species of tsetse, is the major vector of human African trypanosomiasis (HAT) in sub-Saharan Africa. Understanding the population dynamics, and specifically the spatial and temporal stability, of *G. fuscipes* will be important for informing vector control activities. We evaluated spatial and genetic changes over time in twelve populations of the subspecies *G. f. fuscipes* distributed across southeastern Uganda, including a zone of contact between two historically isolated lineages. A total of 861 tsetse flies were genotyped at 16 microsatellite loci and at one mitochondrial locus. Results of an AMOVA indicated that time of sampling did not explain a significant proportion of the variance in allele frequencies observed across all samples. Estimates of differentiation between samples from a single population ranged from approximately 0 to 0.019, using Jost's DEST. Effective population size estimates using

momentum-based and likelihood methods were generally large. We observed significant change in mitochondrial haplotype frequencies in just one population, located along the zone of contact. The change in haplotypes was not accompanied by changes in microsatellite frequencies, raising the possibility of asymmetric mating compatibility in this zone. In conclusion, our results suggest that populations of *G. f. fuscipes* are large and stable over the 8-12 generations studied. Karuma and Kafu are additional areas of overlap of the southern and northern flies' populations. Future studies should aim to reconcile these data with observed seasonal fluctuations in the apparent density of tsetse.

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SANDFLY SURVEILLANCE AND THE DEVELOPMENT OF A LEISHMANIASIS RISK ASSESSMENT IN EAST AFRICA

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The leishmaniases represent a group of neglected tropical diseases found throughout the Horn of Africa (HOA) and East Africa. The current prevalence is largely unknown and underreported. Phlebotomus sand flies are the vectors in the old world. *Phlebotomus orientalis* is the proven vector for visceral leishmaniasis (VL) in Sudan and Ethiopia while *P. martini* is the vector in Kenya and Uganda. Accurate information on sand fly population density, distribution, and species diversity is crucial for the development and implementation of targeted prevention and control efforts. The objective of this study was to assess vector diversity, distribution and relate this to endemic leishmania infection rates in sand flies using GIS mapping techniques. The end state is to develop a surveillance system for detecting and monitoring changes in these variables in East Africa. Sampling was done in five sites in Kenya (Isiolo, Garisa, Wajir, Lamu and West Pokot), and two in Tanzania (Arusha and Kilimanjaro regions).Sand flies were collected using CDC light traps baited with 0.5 kg dry ice from October 2008 to April 2010. Sites in Kenya were visited twice per year while Tanzania once in July 2010 for a period of five nights on each visit. *Leishmania* infections were identified using standard genetic analysis. A conventional Polymerase Chain Reaction (PCR) assay confirmed presence/absence of the parasite and a real-time PCR assay was subsequently run for speciation. Over 20,000 sand flies were collected. A representative sample of 6,843 specimens was identified. *P. orientalis* was found in Isiolo (974), Wajir (328) and Garissa (620) while *P. martini* in Garissa (2) West pokot (78) and Tanzania (17). Sergentomyia species were found in all sites. PCR results are ongoing and will be presented in another forum. The presence of *P. orientalis and P. martini* in Garissa and *P. martini* in Tanzania are the first to be documented. This suggests that there is increasing potential for VL outbreaks in areas of East Africa considered to be low risk.

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NIGERIA *ANOPHELES* VECTOR DATABASE: A REVIEW OF 100 YEARS' RESEARCH

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Anopheles mosquitoes are important vectors of malaria and lymphatic filariasis (LF) which are major public health diseases in Nigeria. Malaria is caused by infection with a protozoan parasite of the genus *Plasmodium* and LF by the parasitic worm *Wuchereria bancrofti*. Knowledge of the *Anopheles* vectors that transmit these diseases is necessary in order to plan vector control appropriately in Nigeria. To present a comprehensive report on the spatial distribution and composition of these vectors, all published data available were collated into a database. Details recorded for each source were the locality, latitude, longitude, time/period of study, species, abundance, sampling and collection methods, morphological and molecular species identification methods, insecticide resistance status, including evidence of the KDR allele, and *Plasmodium falciparum* sporozoite rate and *W. bancrofti* microfilaria prevalence. This collation resulted in a total of 107 publications, encompassing 481,661 vector *Anopheles* mosquitoes in 628 spatially unique descriptions at 145 geo-referenced locations being identified across Nigeria from 1912 to 2010. Overall, the highest number of vector species reported included *An. gambiae* complex (65.6%), *An. funestus* complex (17.5%), *An. gambiae* s.s. (6.4%). *An. arabiensis* (5.0%) and *An. funestus* s.s. (2.3%), with the molecular forms *An. gambiae* M and S identified at 120 locations. A variety of sampling, collection and species identification methods were used over time with an increase in molecular techniques in recent decades. Insecticide resistance to pyrethroids and organochlorines was found in the main *Anopheles* species across 45 locations. Presence of *P. falciparum* and *W. bancrofti* varied between species with the highest sporozoite rates found in *An. gambiae* s.s, *An. funestus* s.s. and *An. moucheti*, and the highest microfilaria prevalence in *An. gambiae* s.l., *An. arabiensis*, and *An. gambiae* s.s. This comprehensive geo-referenced database provides an essential baseline on *Anopheles* vectors and will be an important resource for malaria and LF vector control and elimination programmes in Nigeria.

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HABITAT ASSOCIATIONS OF EASTERN EQUINE ENCEPHALITIS IN THE FLORIDA PANHANDLE

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Eastern Equine Encephalitis virus (EEEV) is an alphavirus with high pathogenicity in both humans and horses. In 2010, Florida had four human fatalities of EEEV and Florida continues to have the highest amount of human cases in the USA. Furthermore, Florida sees year-round EEEV transmission, whereas other states have a more pronounced seasonal pattern. Florida's habitat is uniquely different from the other 48 continental states in that it has both tropical and sub-tropical regions. There are higher levels of EEEV transmission in the panhandle and northern regions of Florida as compared to the central and southern areas. To determine which habitats play a role in EEEV transmission in the Florida panhandle, 24 sentinel sites were categorized as enzootic, periodic, and negative based on the number of chicken seroconversions to EEEV from 2005-2009. The average EEEV prevalence rate in the sentinel chickens across all sites over the last five years was 3.4%, with the most active site's sentinel flock averaging a prevalence rate of 11.95%. A habitat analysis was conducted using Arc GIS 9.3 on all 24 sites, using level two land cover classifications. The land classification data was analyzed using an analysis of variance and comparisons were made between enzootic, periodically enzootic, and negative sites. The analysis of variance produced results showing both risk and protective ecological factors for EEEV transmission. The ecological risk factor found to be associated with higher levels of EEEV transmission was the tree plantation habitat. Protective ecological factors associated with reduced levels of EEEV transmission were vegetated non-forest wetland and wetland coniferous forest habitats. In identifying tree plantations as a habitat of risk, surveillance programs can target these areas for monitoring and treatment, thereby potentially reducing the risk of EEEV in both the human and horse populations within Florida.

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CHARACTERIZATION OF PERITROPHINS FROM THE SAND FLY *PHLEBOTOMUS PAPATASI*

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The sand fly peritrophic matrix (PM) plays a key role in compartmentalization of the blood meal and as barrier to *Leishmania*. However, little is known about sand fly PM molecular components and structural organization. We characterized three peritrophins (PpPer1, PpPer2, and PpPer3) from Phlebotomus papatasi. PpPer1 and PpPer2 display, respectively, four and one chitin-binding domains (CBDs). PpPer3 on the other hand has two CBDs, one mucin-like domain, and a putative domain with hallmarks of a CBD, but with changes in key amino acids. Temporal and spatial expression analyses show that PpPer1 is expressed specifically in the female midgut after blood feeding. PpPer2 and PpPer3 mRNAs were constitutively expressed in midgut and hindgut, with PpPer3 also being expressed in Malpighian tubules. PpPer2 was the only gene expressed in developmental stages. Recombinant PpPer1, PpPer2 and PpPer3 were obtained and shown to display similar biochemical profiles as the native proteins. Our data indicate that rPpPer1 and rPpPer2 are able to bind chitin, suggesting they are involved in PM formation, and likely are also involved in heme detoxification based on their ability to bind heme. In contrast, the mucin-like PpPer3 appears to be involved in protecting the midgut epithelia, and is only expressed in the pyloric triangle. PpPer1 and PpPer3 expression are regulated by Le. major infection. Interestingly, knock down of PpPer1 led to 45% reduction in mRNA levels and 44% in protein which resulted in increases of parasite load of 39% at 48h and 22% at 96h post-infection. The results support the role of PpPer1 as a component of the PM scaffold and may strongly suggest that PpPer1 significantly contributes to the PM overall structure organization and porosity. PpPer1 appears to be a key determinant of the PM role as a barrier to *Leishmania*.

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BURROWING SCABIES MITES ALTER SKIN CELL GENE EXPRESSION

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We have previously demonstrated that the ectoparasitic mite, *Sarcoptes scabiei,* is the source of substances that modulate the cytokine secretion and adhesion molecule expression of host skin cells including epidermal keratinocytes, dermal fibroblasts and microvascular endothelial cells. We have also shown that live mites burrowing into the surface of a human skin equivalent (keratinocytes and fibroblasts in a collagen matrix; HSE) induce the secretion of a variety of both pro- and anti-inflammatory cytokines. Among the most significantly induced cytokines were interleukin-1α (IL-1α), IL-1β, IL-1 receptor antagonist (IL-1ra), IL-6, IL-8, T cell-attracting chemokine (CTACK), monocyte chemoattractant protein-1 (MCP-1), and macrophage- and granulocyte/macrophage colony-stimulating factors (M-CSF and GM-CSF). In this study, we sought to determine if mite burrowing was also able to elicit parallel changes in gene expression by these skin cells. Live scabies mites were allowed to burrow into HSEs for 48 hrs then tissues were frozen at -80°C in RNA*later.* RNA was extracted and subjected to gene expression profiling using Affymetrix GeneChip® Human Gene 1.0 ST microarrays at the Wright State University Center for Genomics Research. Genes for several cytokines were among the most up-regulated by live mites. The gene ontology groups comprised of genes involved in IL-1/IL-1ra activity, tumor necrosis factor production (TNF), IL-6, and vascular endothelial growth factor (VEGF) regulation and production represented 4 of the top 6 groups modulated by live scabies mites. These data indicate that the cytokine secretion induced by live mites burrowing into HSEs is regulated at the gene expression level.

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EVALUATION OF ULTRA LOW VOLUME AND THERMAL FOG PESTICIDE APPLICATIONS AGAINST OLD WORLD PHLEBOTOMINE SAND FLY VECTORS OF *LEISHMANIA* IN KENYA

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One component of the Department of Defense (DoD) pest management system is ultra-low volume (ULV) and/or thermal fog aerosol pesticide application. Despite widespread implementations of this and other components of the system, such as use of repellents and permethrin, US military operations in hot-arid regions still face substantial impacts from insect vectors of disease such as mosquitoes and sand flies. Few studies have compared ULV and thermal fog technologies, and no study has analyzed their performance or efficacy against sand flies in hot-arid environments. In this study we evaluated the Grizzly ULV (Clarke) and the Swingfog SN101E (Swingtec) calibrated on site with two pesticides, Fyfanon (malathion) and Duet (sumithrin, prallethrin, and PBO), in separate trials against caged sentinel *Phlebotomus duboscqi* sand flies and wild populations of *Phlebotomus* and *Sergentomyia* spp. sand flies in the hot-arid North Rift Valley, Kenya. Wild sand fly populations were sampled throughout the study and for all trials sentinel sand flies were arranged in 25-cage grids with five offsite control cages. Spray plots for both the sprayers and chemicals were reciprocated and spray times and environmental conditions were reasonably consistent across trials. Wild sand fly population sampling showed good control in all treated plots as well as a possible repellent effect indicated by increased populations in nearby untreated areas. Wind shear effect was observed in spatial mortality patterns in thermal fog applications, but was notably absent in mortality from concurrent ULV applications. Prior trials with the Grizzly in Kenya demonstrated widespread control with Duet, but the reverse was seen in the present study. Duet applied with the Swingfog provided rapid and widespread control despite sub-optimal conditions, although uneven terrain led to longer spray time in that instance. Prior studies in hot-arid areas in California had shown thermal fog applications superior to ULV when using Fyfanon against mosquitoes, but the present trials showed the reverse against sand flies.

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DIVERSITY AND COMPOSITION OF ANTHROPOPHILIC SPECIES OF *ANOPHELES* (*DIPTERA: CULICIDAE*) IN TWO MALARIA ENDEMIC AREAS OF COLOMBIA

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A central theme in community ecology is the understanding of the factors driving species composition, diversity and variation. It is more important to determine how diversity and composition vary among sites than the number and identity of species in a given site. Therefore, we sought to provide updated information on the diversity, composition and geographical distribution of anthropophilic *Anopheles* species in six localities in Colombia, in the Urabá-Bajo Cauca-Alto Sinú (UCS) and Pacific (P) regions. Each locality was visited four times, every three months,

from November 2008 to June 2010. Mosquitoes were collected for five consecutive nights (18:00-24:00 h), and one additional night (18:00 to 6:00 h). A total of 9,839 specimens belonging to 10 species were collected. Relative abundance showed a reverse-J-shaped curve with few dominant and many rare species. In the overall survey, *An. nuñeztovari* s.l. and *An. darlingi* were the most abundant (47.21% and 40.47%, espectively). *An. punctimacula* and *An. neivai* (<0.01%) were the least bundant. *An. nuneztovari* s.l. predominated mainly in UCS (84.53%), whereas *An. darlingi* dominated in P (55.14%). Other species were found in only one area and in low abundance; *An. pseudopunctipennis* (4.72%), *An. albitarsis* s.l. (2.62%) and *An. triannulatus* s.l. (1.96%) in UCS, and *An. calderoni* (10.63%) and *An. albimanus* (3.63%) in P. UCS had the highest anthropophilic anopheline diversity. Diversity values at different spatial and temporal scales showed statistically significant differences, suggesting that anopheline communities present complex dynamics. In general, the composition, abundance and diversity of these anophelines were highly variable; therefore, control programs should be adapted to the characteristics of each community for maximal efficiency.

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TECHNIQUE FOR PRESERVATION OF MICROFILARIAE OF *WUCHERERIA BANCROFTI*

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Five different types of anti-coagulant, namely, heparin, ACD, ACD-D5, CPD and CPDA-1, were studied for their abilities in preserving microfilariae of *Wuchereria bancrofti* in blood collected from infected patients. The rates of infection generally decreased as the duration of preservation increased. There was no infection detected in mosquitoes fed with blood containing heparin, ACD and ACD-D5 on day 8, day 10 and day 9 after blood collection, respectively. As for mosquitoes fed with blood containing CPD and CPDA-1, infections occurred even the blood used was 10 days old after collection. The responses of mosquitoes to feeding of patients' blood with five different anti-coagulant formulae did not differ significantly on each day from day 3 to day 10 after collection ($P>0.05$). The average numbers of the third infective stage larvae of *W. bancrofti* per mosquito fed with patients' blood with five different anti-coagulant formulae ranged from 0.3 to 3.4 larvae per mosquito.

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A SURVEY OF *ANOPHELES* SPECIES IN A MALARIA ENDEMIC AREA ALONG THE THAI-MYANMAR BORDER

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Anopheles larval mosquitoes were surveyed from the stream in Kanchanaburi Province, western Thailand over a two-year period. Three major groups were morphologically identified, including Minimus subgroup and two other related species (75.74%), Maculatus group (20.47%), and Barbirostris group (0.48%). The other 116 specimens were morphologically identified as *An. culicifacies* (3.05%), *An. philippinensis* (0.17%), and *An. vagus* (0.09%). Based on a molecular identification assay, 2 species within the Minimus subgroup were identified, *An. minimus* (69.93%) and *An. harrisoni* (0.06%) and 2 genetically related species within the Aconitus subgroup, *An. aconitus* (0.63%) and *An. varuna* (5.13%) were described. The Minimus subgroup and other related species were more prevalent during the dry season (52.58%) compared to the hot and rainy seasons. In general, number of *Anopheles* larvae collected from the stream was significantly higher in the second year compared to the first year. This study suggested that site-specific studies should be conducted to accurately determine vector larval habitats and adult activity patterns and linking their importance in malaria transmission in a given area.

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INSIGHTS IN *WOLBACHIA* - TSETSE (GENUS *GLOSSINA*) SYMBIOTIC INTERACTIONS

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Wolbachia is a genus of endosymbiotic α-Proteobacteria infecting a wide range of arthropods and filarial nematodes. *Wolbachia* is able to induce reproductive abnormalities such as cytoplasmic incompatibility (CI), parthenogenesis, feminization and male killing, thus affecting biology, ecology and evolution of its hosts. The bacterial group has prompted research regarding its potential for the control of agricultural and medical disease vectors, including *Glossina* sp., which transmits African trypanosomes, the causative agents of sleeping sickness in humans and nagana in animals. In the present study, we employed a *Wolbachia* specific *16S rRNA* PCR assay to investigate the presence of *Wolbachia* in six different laboratory stocks as well as in natural populations of eleven different *Glossina* species originating from 11 African countries. *Wolbachia* was prevalent in *Glossina morsitans morsitans*, *G. morsitans centralis* and *G. austeni* populations. It was also detected in *G. brevipalpis*, and, for the first time, in *G. pallidipes, G. palpalis gambiensis, G. p. palpalis and G. medicorum*. On the other hand, *Wolbachia* was not found in *G. fuscipes fuscipes*, *G. m. submorsitans* and *G. tachinoides*. *Wolbachia* infections of different laboratory and natural populations of *Glossina* species were characterized using *16S rRNA*, the *wsp* (Wolbachia Surface Protein) gene and MLST (Multi Locus Sequence Typing) gene markers. This analysis led to the detection of horizontal gene transfer events, in which at least four *Wobachia* genes (*16S rRNA, ftsZ, fbpA* and *wsp*) were inserted into the tsetse fly nuclear genome. In addition, it was shown that *G. m. morsitans* males present higher *Wolbachia* load than females. *Wolbachia* infections were detected in both laboratory and natural populations of several different *Glossina* species. The characterization of these *Wolbachia* strains promises to lead to a deeper insight in tsetse-*Wolbachia* interactions, which is essential for the development and use of *Wolbachia*-based biological control methods.

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SPECIES COMPOSITION OF THE MOSQUITO FAUNA OF WESTERN UGANDA

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The mosquito species composition for several locations in Uganda was described during routine arboviral surveillance and outbreak investigations from the mid 1930s to the early 1970s. During that period, mosquito species of Bundibugyo district (then Bwamba County), where Semliki Forest is located, were investigated in detail and over 160 mosquito species were described in this region. Civil instabilities in the 1970s and 1980s halted routine arboviral disease investigations and mosquito species records in Uganda have not been updated for more than 40 years.

During recent arboviral surveillance and zoonotic disease investigations in western Uganda conducted by Uganda Virus Research Institute and the US Centers for Disease Control and Prevention (CDC), mosquitoes were collected in five locations in western Uganda: Sempaya, in Semliki Forest, Kibale Forest, Bwindi Impenetrable Forest, and Mweya and Maramagambo Forest in Queen Elizabeth National Park. Seventy-three species were identified in Sempaya including five species described in Bundibugyo District for the first time: *Aedes (Stegomyia) aegypti formosus* (Walker), *Aedes (Stegomyia) metallicus* (Edwards), *Anopheles (Cellia) rivulorum* Leeson, *Uranotaenia (Uranotaenia) chorleyi* Edwards and *Uranotaenia (Uranotaenia) pallidocephala* Theobald. Twenty-eight mosquito species were identified in Kibale Forest, 41 in Bwindi Impenetrable Forest, 36 in Mweya and 51 in Maramagambo Forest. This is the first description of the mosquito fauna for these four locations. Mosquito species composition and the implication for arboviral transmission for these five locations will be discussed.

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TARGETING THE *PHLEBOTOMUS PAPATASI* PPCHIT1 AS A STRATEGY TO CONTROL SAND FLY-TRANSMITTED LEISHMANIASIS

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For sand flies, the peritrophic matrix constitutes a barrier against *Leishmania* infection. Recently, we have demonstrated that this barrier could be reinforced by knocking down PpChit1, a midgut-specific chitinase of the sand fly *Phebotomus papatasi*, pointing to PpChit1 as a target for paratransgenesis and transmission blocking vaccine approaches. As anti-PpChit1 antibodies are capable of neutralizing chitinolytic activities in midgut extracts not only of *P. papatasi*, but also of *P. argentipes*, PpChit1 may be a target in cross-species strategies against leishmaniasis. In order to assess the potential of anti-PpChit1 antibodies reducing *Le. major* load in *P. papatasi*, we tested two different anti-PpChit1 antisera: anti-full length and anti-catalytic domain PpChit1antisera. Recombinant VR2001 plasmids encoding full length and PpChit1 catalytic domain were injected in mice ears four times in two weeks intervals. Specificity of such antisera was tested by Western blots against midgut extracts, the respective PpChit1 recombinants, and recombinant peritrophins. The recombinant proteins were produced in CHO-S mammalian cells and purified with Ni-NTA columns. Sand fly infections were performed with 5 x 10^6 amastigotes/ml along with neat anti-PpChit1 serum or pre-immune serum. Contrasting to our previous results on PpChit1 knock down via RNAi, feeding on anti-full length PpChit1 antiserum increased *Le. major* load in *P. papatasi* midguts. As this antiserum cross-reacted with other midgut extract proteins displaying the expected sizes of peritrophins and with the recombinant peritrophins, the cross-reactivity may be affecting *Le. major* development in an unexpected manner. Antiserum against the PpChit1 catalytic domain, on the other hand, recognized a single band in midgut extracts and the recombinant catalytic domain of PpChit1, suggesting it specifically recognizes PpChit1. We are currently testing if this antiserum can reduce *Le. major* load in *P. papatasi*. Future work is planned to assess if anti-PpChit1 catalytic domain antiserum also can reduce transmission to naïve hosts.

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CONTAMINATION OF *ANOPHELES ARABIENSIS* WITH PYRIPROXYFEN USING ODOR BAITED STATIONS

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Mosquito larviciding is expected to play a key role in malaria control strategies alongside targeting the adult vectors and malaria parasite. It was recently shown that vectors of Dengue fever can be used to disseminate pyriproxyfen (PPF), a juvenile hormone analogue, into their own breeding sites. For this process to be successful, wild mosquitoes must pick up the larvicide and retain it until reaching a breeding site, where during the oviposition process, they contaminate the water body. This study aimed to adapt the technique for malaria vectors in rural Tanzania. Odor baited stations (OBS) have been tested in this area and were successful in attracting large numbers of wild mosquitoes. PPF powder was applied to the eave-baffles of OBS which served as contamination sites for wild mosquitoes. Three trap nights were conducted and each morning the mosquitoes were collected from exit traps mounted on the OBS. Species collected consisted of both Anophelines and Culicines. These mosquitoes are known to share breeding sites in the dry season in this area, making them potential PPF carriers for malaria control. Collected mosquitoes were then killed and dipped in cups of water containing 10 *Anopheles arabiensis* larvae. Reduction in mosquito emergence was observed from these cups and emergence decreased as the number of contaminated mosquitoes was increased in the cups. Further studies are being carried out to test whether cows can be sprayed with PPF in order to contaminate zoophagic mosquitoes during blood feeding.

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IRON-BINDING PROTEINS OF THE SCABIES MITE *SARCOPTES SCABIEI* AND THEIR IMPLICATIONS FOR INFECTION

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Scabies is a neglected parasitic disease with debilitating effects for both humans and animals. Secondary infections with bacteria are common in crusted scabies patients, particularly in remote Aboriginal communities. It has been reported that pigs can develop auto-antibodies to transferrin during infection with *Sarcoptes scabiei*, where the majority of pigs infected with *S. scabiei* tested positive for both IgG and IgM antibodies to transferrin. Research suggests that this may also be the case for human scabies patients. Recognition of commercial human transferrin by human IgG has been shown by preliminary ELISAs for both crusted and ordinary scabies. It is presumed that a similar mechanism is involved for the production of auto-antibodies in both humans and pigs. A *S. scabiei* var. *hominis* EST database was used to screen for possible iron-binding homologues to transferrin and ferritin. Transferrin was expressed in bacteria as two single domains, N- and C-terminals. Ferritin was expressed as a whole protein. Bioinformatic analysis revealed that mite transferrin has lost many of the conserved residues required for binding iron at both terminals. A pig model was developed by scientists at QIMR for human scabies research. Analysis of recombinant proteins by ELISA indicated that mite iron-binding proteins are antigenic to experimentally infected pigs but not naïve pigs. Competition ELISAs suggest that the antibody response to host proteins may be the result of cross-reactivity with mite epitopes. Further testing using human antibodies to detect recombinant mite proteins is required to determine their antigenicity to humans. The possible loss of iron-binding ability by mite transferrin may have implications for the infection process in both humans and animals.

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ARTIFICIAL LIGHT INCREASES HOUSE INFESTATION BY NON-DOMICILIATED *TRIATOMA DIMIDIATA*

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Triatoma dimidiata is a major vector of Chagas disease and we previously documented the seasonal infestation of houses by this species in the Yucatan peninsula. We also found that bugs were specifically attracted to houses, but the factors mediating this attraction remained unclear. Artificial light has been known for a long time to attract many insect species and light traps have been used to collect different species of triatomines, including *T. dimidiata*. Several authors have also suggested that light might attract *T. dimidiata* to houses, but the role of artificial light in house infestation has never been clearly demonstrated or quantified. Here we performed an exhaustive spatial analysis of house infestation pattern by *T. dimidiata* in relation to the distribution of artificial light sources in three different villages from the Yucatan peninsula. In all three villages, infested houses were on average significantly closer to artificial light sources than non-infested houses, and public lights rather than domestic lights were associated with house infestation. Thus, houses closer to a public street light were 2.72 times more likely to be infested that houses further from public lights (OR, CI95% 2.04-3.61). Behavioural experiments using a dual-choice chamber further confirmed that adult *T. dimidiata* is attracted to white light during its nocturnal activity and in a dose-response manner. While public lighting is usually associated with increased development, these data clearly show that it also directly contributes to house infestation by non-domiciliated *T. dimidiata*.

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CORRELATION OF ANTIBODY AVIDITY WITH DENGUE DISEASE SEVERITY IN HUMAN SERUM SAMPLES

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Dengue virus (DENV) continues to be a major health problem in tropical and subtropical regions worldwide. A secondary infection with a different DENV serotype is a risk factor for severe disease, and the humoral and cellular immune response have been implicated in both protection and pathogenesis. Avidity is a measure of the overall strength of antibody-antigen interaction and depends on the number and affinity of individual binding sites. We hypothesized that low-avidity anti-DENV antibodies could be associated with greater occurrence of symptomatic infection or more severe disease. We are analyzing well-characterized serum samples from a hospital-based study of pediatric dengue in Managua, Nicaragua, ongoing since 2005. Serum avidity was measured using a modified competition ELISA protocol. Purified viral particles (Nicaraguan strain DENV2 N172) were used as antigen. Serum from primary and secondary DENV infections diluted in blocking buffer were pre-incubated with DENV2 for 1 hour ("competition"), then virus-coated wells were incubated with the serum-virus mixtures, followed by the secondary antibody and substrate. For each plate, background values were subtracted, then the percent of inhibition of antibody binding was calculated by subtracting the ratio of the adjusted OD after virus competition from the adjusted OD without virus competition. Plates were normalized using WHO anti-DENV polyvalent reference serum. The assay was validated by confirming higher avidity IgG antibodies in DENV-immune serum against viral particles of the same serotype in comparison to DENV viral particles of a different serotype. Also, avidity was greater in cases of secondary DENV infections compared to primary infections, as expected. Preliminary results of analysis of secondary DENV2 cases from the hospital-based study (dengue fever, n=12; dengue hemorrhagic fever, n=10; dengue shock syndrome, n=13) revealed a trend of inverse correlation between serum avidity and disease severity. This trend was also detected using a different avidity ELISA protocol with urea washes. These results are being correlated with homotypic and heterotypic neutralization/enhancement titers. Overall, these studies should help define antibody characteristics associated with mild versus severe dengue disease useful for vaccine development as well as furthering our understanding of dengue pathogenesis.

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IN COLOMBIAN OUTPATIENTS WITH CLINICAL SUSPICION OF DENGUE FEVER, HOST BIOMARKERS DIFFERENTIATE BETWEEN SUBJECTS WITH CONFIRMED DENGUE FEVER AND LEPTOSPIROSIS

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Dengue represents the most important arboviral infection worldwide and is of increasing global importance. Causes of fever are often non-specific. Acute febrile syndromes like dengue fever and leptospirosis have overlapping geographic distributions, similar clinical presentations and potentially life-threatening complications, but require different treatments. This study was undertaken to determine if perturbations in host biomarkers can differentiate between individuals with dengue fever and leptospirosis during the acute phase of illness. We randomly selected subjects from a prospective cohort study of acute febrile illness in Bucaramanga, Colombia and tested 18 serum biomarkers by ELISA in individuals with dengue fever (DF, n=112) and leptospirosis (n=47). Biomarkers were selected for further analysis if they had good discriminatory ability (area under the ROC curve (AUC) >0.80) and were beyond a reference range (assessed using local healthy controls). We identified 5 candidate biomarkers (Ang-like 3, IL-18 binding protein (IL-18BP), IP-10, sICAM-1, sEndoglin) that were dichotomized (based on the Youden index) to create a score ranging from 0-5. Using this biomarker score, we could discriminate between dengue and leptospirosis with an AUC of 0.96 (95% CI, 0.93-0.99). In order to generate a more parsimonious biomarker score, we took the 2 biomarkers with the best discriminatory ability (AUC >0.90, IL-18BP and sEndoglin). We added the 2-biomarker score to an easy-to-measure bedside index consisting of the 3 clinical predictors with the best discriminatory ability (assessed using forward step-wise regression): sore throat, facial erythema and hepatomegaly. The bedside index on its own had moderate discriminatory ability (AUC, 95% CI; 0.74, 0.67-0.81) whereas a bedside index in conjunction with biomarkers had excellent discriminatory ability (AUC, 95% CI; 0.95, 0.90-0.98), and was significantly better than the bedside index alone ($p<0.0001$, Method of Delong et al.). In conclusion, these results suggest that host biomarkers may have utility in differentiating dengue fever from leptospirosis.

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CHARACTERIZING INTRA-HOST DIVERSITY OF DENGUE VIRUS POPULATIONS AND HOST IMMUNE EFFECTOR REPERTOIRES IN HUMAN DENGUE VIRUS INFECTIONS

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Dengue virus (DENV), a positive-strand RNA *Flavivirus*, causes tens of millions of cases of dengue annually, with three billion people at risk for infection worldwide. The clinical manifestations of dengue range from subclinical infection to life-threatening syndromes, and despite intense research efforts, precise causes for the observed range in disease severity remain unclear. We are employing high-throughput sequencing to investigate the contribution of diversity associated with viral populations and host immune effectors to DENV evolution and the course of dengue pathogenesis. This study utilizes samples from two long-term studies of pediatric dengue in Nicaragua, accompanied by extensive supporting clinical and epidemiological data. Our DENV sequencing efforts are the first to showcase the diversity landscape across the entire DENV genome in human samples and are revealing relationships between INTER-host diversity (i.e., across individuals and epidemics) and INTRA-host diversity (i.e., within an individual), with implications for the study of DENV evolutionary dynamics. We are also simultaneously assessing longitudinal differences in B cell diversity, as assessed by immunoglobulin heavy chain rearrangements ("*IgH*"), for an unbiased evaluation of the clonality of infection-associated antibodies in blood during DENV infection. We have identified expansion of B cells with specific *IgH* in unsorted PBMCs during primary and secondary DENV infections, with clones from this initial cohort appearing earlier for patients who present with secondary infections, as would be expected if there were contributions from pre-existing immunity. We hope that our analyses of samples for which we have both types of datasets available will allow us to discern correlations between hotspots for viral diversity (or immutable regions), and specificity of the antibody response (i.e., expansion of specific B cell clones, as assessed by diversity of B cell-associated *IgH*). Such correlations may facilitate assessment of whether regions of diversity or regions of immutability serve as potential protein epitopes for engaging B cell-directed immunity.

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CHARACTERIZATION OF THIRD AND FOURTH DENGUE VIRUS INFECTIONS IN A PEDIATRIC COHORT STUDY

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Four dengue virus serotypes (DENV1-4) circulate globally, causing more human illness than any other arthropod-borne virus. DENV infection results in inapparent infection, Dengue Fever, life-threatening DHF/DSS with fluid loss and hypotensive shock, or other severe manifestations. The immune response to DENV protects against re-infection with the same serotype; however, the greatest risk factor for severe disease is prior infection with a heterologous DENV serotype. Yet, little information exists specifically about 2nd vs 3rd vs 4th DENV infections, which are all classified as secondary (2°) infections. A cohort study of dengue established in Managua, Nicaragua, in August 2004 is now in its 7th year, following 3,700 children 2-14 years old, with ~5% annual loss to follow-up. Participants are encouraged to come to the study health center at first sign of illness, and 94% of febrile illnesses present within 72 hours of symptom onset. A healthy annual blood sample is collected, and paired annual samples are examined on a yearly basis to identify new primary and 2° infections. Primary infections are defined as seroconversions and 2° infections as a $\geq$4-fold increase in anti-DENV antibody (Ab) titers, determined by Inhibition ELISA. Using data from 7 dengue seasons and 6 annual sample collections, we identified 16 children who entered the cohort DENV-naïve and experienced 3 documented DENV infections; 103 who entered with anti-DENV Abs and had 2 additional DENV infections, and 4 who entered with anti-DENV Abs and had 3 documented infections. We are currently investigating these repeat infections in serial annual serum samples by determining the endpoint neutralizing titer (NT_{50}) to the 4 DENV serotypes using a flow cytometry-based neutralization assay. A separate analysis of DENV2 DHF/DSS cases in the cohort revealed that the majority (5/9; 56%) had evidence of multiple prior infections (substantial NT_{50} titers to DENV1 and DENV3); in contrast, only 6/29 (21%) of DF cases caused by DENV2 had high DENV1 and DENV3 titers prior to infection. The results of confirmed 3rd and 4th infections in relation to infection outcome (symptomatic vs inapparent), disease severity, interval between infections, and sequence of DENV serotypes will be presented. Further studies will compare these results to those obtained in 2nd DENV infections. These data should advance our specific knowledge of repeat DENV infections, with implications for vaccine design.

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ACUTE TRANSAMINITIS DURING DENGUE FEVER ILLNESS

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Dengue fever is endemic to Singapore, where it mainly affects the adult population. Acute transaminitis, or increase in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) liver enzyme levels, has commonly been observed in dengue patients during early illness. However, there are few studies showing the relationship between acute transaminitis and disease severity. 699 dengue PCR positive cases seen at Tan Tock Seng Hospital in Singapore from 2006 to 2008 were retrospectively reviewed, and demographic, clinical, laboratory, and disease outcome data were extracted. Statistical analyses were performed to show any association between AST or ALT and disease outcomes. 86.4% of cases had AST levels greater than the upper limit of normal (ULN), and 46.4% had ALT above ULN. Only 10 patients had AST or ALT >1000 U/L, fulfilling the 2009 World Health Organization (WHO) definition of severe liver impairment. Median AST was 92 U/L for non-severe dengue cases and 125 U/L for severe dengue patients (WHO 2009 classifications), excluding those with isolated transaminitis--one of the severe dengue criteria ($p<0.005$); median ALT values were 52 and 74 U/L ($p<0.005$). AST and ALT values also increased significantly in conjunction with disease severity under the WHO 1997 classification system. 3 patients required intensive care, and 1 died. AST or ALT values did not correlate with hospital length of stay (Spearman's rho=-0.02 and -0.03; p=0.59 and 0.37, respectively). Regression analysis showed that determining an AST or ALT cutoff to predict severe dengue was difficult since the area under the receiver operating characteristic (ROC) curve was 0.62 for AST and 0.59 for ALT. Most of the dengue cases in this study experienced transient transaminitis. There was a significant overall association of higher AST and ALT levels with severe disease outcomes, but there was no good cutoff level to predict severe dengue.

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SPATIOTEMPORAL PATTERNS OF *AEDES AEGYPTI* MOSQUITO POPULATIONS IN CAIRNS: ASSESSING THE DRIVERS OF RISK

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Understanding the dynamics of the primary dengue vector, *Aedes aegypti*, is vital to controlling the disease. Current monitoring in Cairns, Australia, involves trapping *Ae. aegypti* mosquitoes in sticky Ovitraps, with weekly mosquito counts determining whether adult mosquito abundance is within expected limits. This study aimed to determine relationships between *Ae. aegypti* and environmental drivers: rainfall, temperature and humidity. Data from sticky Ovitraps traps in Cairns were collected for the period 2007-2010. Climate data (rainfall, temperature and humidity) for Cairns were accessed via the Bureau of Meteorology for the same period. Exploration of the data was undertaken using Spearman's rank correlation and Poisson regression. Results showed positive correlations between *Ae. aegypti* abundance and maximum weekly temperature (rho = 0.5342), total weekly rainfall (rho=0.3178) and average weekly humidity (rho=0.3844). Maximum weekly temperature was a statistically significant predictor of *Ae. aegypti* abundance (p= 0.000). The expected change in log count for a one degree Celsius increase in temperature was 0.1830. Total weekly rainfall was also a statistically significant predictor of *Ae. aegypti* abundance (p= 0.028). The expected change in log count for a one millimeter increase in rainfall was 0.0020. Interestingly, humidity was not a significant predictor of *Ae. aegypti* abundance (p= 0.0700). Dengue is an emerging arbovirus that causes considerable morbidity and mortality in the Asia-Pacific region. This study contributes information on the influence of environmental drivers (rainfall, temperature and humidity) on *Ae. aegypti*, the primary dengue vector. Findings suggest that control of the vector should be focused on climatic factors because abundance of *Ae. aegypti* is associated with these conditions. This information can be used with other data to predict the risk of dengue in a given geographic location.

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SAFETY PROFILE OF THE CYD LIVE, ATTENUATED, TETRAVALENT, DENGUE VACCINE

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A tetravalent dengue vaccine (TDV) comprising 4 recombinant, live, attenuated viruses, (CYD-1-4), given with a 3-dose regimen over 12 months, is currently in clinical phase 3 evaluation. We performed an integrated analysis of safety of all 13 trials (3 phase I, 5 completed phase II, and 5 ongoing phase II trials) conducted to date with the current TDV formulation, in both dengue-endemic and non-endemic areas. Data were analyzed by treatment given for unblinded trials (TDV, placebo, or control vaccine) or in a blinded manner for ongoing trials, and by age group: adults (>/=18 yrs), adolescents (12-17 yrs), children (2-11 yrs), and toddlers (<2 yrs). After the 1st, 2nd and 3rd vaccinations respectively, the TDV analysis set comprised 890, 710 and 555 participants, and the blinded analysis set comprised 6782, 5588, and 3897. After the 1st TDV dose, 21.3% of adults, 24.2% of adolescents, 25.4% of children and 18.3% of toddlers had solicited injection site reactions. These percentages ranged from 20-35% after the 2nd, and 17-30% after the 3rd dose, respectively. In comparison these rates after the 1st injection ranged from 27-56% after active control vaccination, 7-19% after placebo, and 17-37% in the blinded dataset. Solicited systemic reactions after the 1st TDV dose affected 56.2% of adults, 63.7% of adolescents, and 45% of children and toddlers, decreasing to 28-48% after the 2nd, and 20-37% after the 3rd. In comparison, after the 1st injection these rates ranged from 39-73% after active control vaccination, 29-49% after placebo, and 40-55% in the unblinded dataset. Overall, headache was the most frequent solicited systemic reaction after any TDV dose. Most solicited systemic reactions were mild and lasted 1-3 days. The incidence of serious adverse events was similar in the TDV group compared with other groups. Reactogenicity was no higher in children than adults, and no higher after the 2nd or 3rd doses compared with the 1st. Based on all available data, TDV has a satisfactory safety and reactogenicity profile, comparable to that of the control vaccines.

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2011 UPDATE ON THE SANOFI PASTEUR CYD TETRAVALENT DENGUE VACCINE: INITIATION OF CLINICAL PHASE 3 PROGRAM

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While no licensed vaccine is available against dengue, the sanofi pasteur 2nd generation candidate, in development since 1998, has reached industrial scale-up, clinical phase III, and has been given to more than 6000 children and adults. This candidate, a mixture of 4 recombinant, live attenuated viruses (CYD-1-4) at 10^5 $CCID_{50}$/virus, is being tested with a robust 3-dose, 0-6-12 month regimen. Industrialization efforts include the construction of 3 new dedicated facilities (Utilities, QC and Production) at a new vaccine production site in Neuville-sur-Saone, France. Banking systems for serum-free Vero cells have been established to produce master and working viral seeds and cells, providing reliable and consistent supply, a production process with no raw materials of animal origin, and a vaccine with no preservatives, adjuvants, or antibiotics. To further characterize the vaccine's safety, a biodistribution and shedding study was performed in cynomolgus monkeys, showing that there was no neurotropism, no shedding, and only limited viscerotropism; CYD viruses were limited mainly to the injection site and the lymph nodes during the first days after injection and were not associated with any toxicological findings other than expected injection site reactions. An integrated safety analysis of all available data from completed phase 1 and 2 studies, and on blinded data from all ongoing phase 2 studies shows a satisfactory safety and reactogenicity profile, comparable to that of control vaccines. The 1st phase 3 trial started in 2010 in Australian adults to test lot consistency. Among several phase 3 trials due to start in 2011, 2 are efficacy studies conducted in 2-16YO children from multiple countries in Asia and Latin America respectively. To prepare the sites for these trials, in particular to assess whether potential dengue cases can be identified and to test the diagnostic algorithm in the field, prospective, active-surveillance studies were initiated in these areas. Results from an ongoing efficacy proof-of-concept trial are expected by the end of 2012.

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IMMUNOGENICITY AND LARGE SCALE SAFETY OF THE LIVE, ATTENUATED, TETRAVALENT, CYD DENGUE VACCINE IN 2-45 YEAR-OLDS IN SINGAPORE

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A tetravalent dengue vaccine (TDV) comprising 4 recombinant, live, attenuated viruses (CYD-1-4) is currently in clinical phase 3 evaluation. In an observed-blinded, age-stratified study, we randomized 1200 volunteers 3:1 to receive 3 subcutaneous doses of TDV or control at Months 0-6-12 (ClinicalTrials.gov: NCT00880893). Controls were placebo for the 1st dose (all ages) and licensed hepatitis A (for <12YO) or influenza vaccine (≥12YO) for subsequent doses. The primary objective was to evaluate dengue virus (DENV) serotype-specific antibody responses before and 28 days after each vaccination in a subset of 600 using a $PRNT_{50}$ assay. Safety of TDV was documented as a co-primary objective. Between Apr and Oct 2009, we enrolled and randomized 317 children (2-11YO), 187 adolescents (12-17YO), and 696 adults (18-45 YO). At baseline ~10% of children/adolescents and ~30% of adults were seropositive (titer ≥10), to at least one serotype. After 3 TDV doses, 66.5% (all ages combined) were seropositive to all 4 serotypes, and 87.2% were seropositive to ≥3 serotypes. Geometric mean titers 28 days after the 3rd dose of TDV (all ages) ranged from 43.0 against DENV1 to 100 against DENV4. Titers were higher against in children than in adolescents. Titers in the control group remained close to baseline levels. The 1st dose of TDV was slightly more reactogenicity compared with placebo. Reactogenicity of subsequent TDV doses was no higher than after the 1st, and was comparable with that of the active control vaccines. One SAE (tension headache secondary to untreated allergic rhinitis 17 days after 2nd dose in a 9YO boy) was reported as possibly-related to TDV by the investigator. The safety profile of CYD TDV in a large cohort of volunteers from Singapore was satisfactory and consistent with observations from earlier trials. Vaccination elicited an immune response against all 4 serotypes in the majority of vaccinees. Some differences in response between age group were noted, possibly reflecting the unique epidemiology of dengue in Singapore.

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ANALYSIS OF *IN VITRO* ENHANCEMENT RESPONSE CURVES AGAINST INFECTED PATIENT AND VACCINEE SERA

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Antibody-dependent enhancement (ADE) has been correlated with increased dengue disease severity. However, measurements of ADE *in vitro* can be dependent on the experimental and analytical parameters used, and their relevance to quantification of ADE and clinical outcome has not been well studied. Here we used pseudoinfectious Dengue virus (DENV) reporter virus particles (RVPs) from each of the four serotypes of DENV to measure and characterize the enhancing response of monoclonal antibodies and sera derived from naturally-infected patients and tetravalent dengue vaccine recipients. Enhancement assays were done in multiple cell types, using various input titers of virus, and under different experimental conditions to derive detailed enhancement curves. Several distinct types of enhancing curves were observed, and a curve fit equation was derived for each type of curve in order to calculate precise enhancement titers. Three distinct metrics were derived from each enhancement curve - titer (peak serum dilution), power (peak height), and polydispersity (peak width) - and the effect of experimental conditions on each metric was assessed to determine the most reliable indicator of *in vitro* enhancement (i.e. independent of experimental conditions). To help determine the clinical relevance of ADE measurements, each measurement was also correlated with neutralization titers derived from each serum (against all 4 serotypes) as well as clinical outcomes of patients and vaccinees. A better understanding of *in vitro* ADE measurements may help quantify the potentially protective and pathogenic immune responses generated against each serotype of DENV within infected and vaccinated patients.

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RISK FACTORS FOR FATALITY AMONG CONFIRMED ADULT DENGUE INPATIENTS IN SINGAPORE: A MATCHED CASE-CONTROL STUDY

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Dengue is an important viral infection affecting most tropical and subtropical areas of the world. Reports of death in adult dengue cases are rare. We conducted a multi-center retrospective study of polymerase chain reaction and non-structural protein 1 (NS-1) confirmed adult (older than 15 years) dengue inpatients in Singapore from 1 January 2004 to 31 December 2008. Initial unmatched analysis showed age was significantly different among fatal cases and non-fatal controls ($p<0.001$). Subsequent analyses matched for age and year of admission to control for different predominant circulating dengue serotypes and yielded 28 cases and 80 controls. World Health Organization 1997 and 2009 criteria were applied to define dengue hemorrhagic fever (DHF), warning signs and severe dengue. Statistical significance was assessed by conditional logistic regression modeling. Versus controls, fatal cases had significantly more comorbid conditions (75% versus 51.3%; $p<0.01$), renal injury defined as serum creatinine more than two times upper limit of normal (71.4% vs. 2.5%; $p<0.001$), warning signs (96.4% vs. 75%; $p<0.05$), severe dengue (100% vs. 22.5%; $p<0.01$), higher median pulse rate (128 vs. 95 per minute; $p<0.001$), alanine transaminase (490 vs 74 Unit/Liter; $p<0.05$) and aspartate transaminase (1158 vs. 112.5 U/L, $p<0.05$) during hospitalization. Leukocyte count and serum protein were significantly lower among cases ($p<0.001$ and $p<0.05$ respectively). There was no statistical significant difference between the prevalence of DHF, median platelet nadir and hematocrit level among cases and controls. The rates of intravenous fluid and platelet transfusion were higher among cases ([92.9% vs. 41.3%; $p<0.05$] and [64.3% vs. 11.3%; $p<0.01$ respectively]). None of the controls were admitted to intensive care unit (ICU) or given blood transfusion, while 71.4% and 28.6% of cases required ICU admission and given blood transfusion. None of the variables was statistically significant in the multivariate analysis. Findings from this study should be validated by larger cohorts.

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ANALYSIS OF DENGUE VIRUS 3 PRM/E MUTANTS TO DETERMINE THE CONTRIBUTION OF EACH RESIDUE TO ENV FUNCTION

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While the functions of Dengue virus (DENV) prM/E are well known, most functional regions of the protein have only been partially mapped. Using Shotgun Mutagenesis technology, a comprehensive plasmid mutation library for DENV-3 prM/E was created in which each prM/E residue was

individually mutated to a defined substitution, expressed in human cells, and analyzed for its effect on viral production and infectivity. This approach expresses each prM/E mutant in mammalian cells that contain the complementary nonstructural proteins required to produce infectious DENV reporter virus particles (RVPs). By looking for expression of a luminescent reporter in target cells, each mutant's ability to support viral infection can be assessed. In total, over 1,000 mutants of DENV prM/E were individually tested for function. Intermediate functions of prM/E, such as viral budding, were also assessed. Structures that are functionally responsible for viral production and infectivity have been mapped in order to better understand how the protein functions. The identification of critical functional structures is expected to help direct the development of therapeutics, diagnostics and vaccines.

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DENGUE FEVER AMONG HOSPITALIZED FEBRILE PATIENTS IN NORTHERN TANZANIA

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Despite its initial description in the tropics at the 17th century, little is known about the prevalence of dengue (DEN) virus infection in sub-Saharan Africa, particularly during non-epidemic periods. To understand the role of DEN as a cause of illness among febrile inpatients, we conducted a one year prospective study in northern Tanzania from September 2007. Serum collected from febrile inpatients from two hospitals was tested for DEN IgM and IgG antibodies using an IgM capture ELISA and Indirect IgG ELISA (both PanBio, Brisbane, Australia) and for DEN and flavivirus by PCR using previously validated primers. Presumptive acute DEN infection was defined as a positive anti-DEN IgM ELISA result. A positive anti-DEN IgG ELISA defined prior flavivirus exposure. Confirmed acute DEN or flavivirus infection was defined as a positive PCR for DEN or flavivirus, respectively. Of 870 participants, DEN IgM serology was performed on 747 (86.0%); 380 (50.1%) were infants and children, 356 (47.7%) were females, and 326 (44%) lived in rural areas. Seventy one (9.5%) had presumptive acute DEN infection; their median (range) age was 14.4(0.3, 95.8) years. DEN IgG serology was performed on 751(86.3%); 384 (51.1%) were infants and children. Of those tested, 80(10.7%) had prior flavivirus exposure. Unlike presumptive acute DEN infection, prior flavivirus exposure was associated with rural residence, (OR 1.8, p-value 0.027) and was less common among infants and children than among adults and adolescents (OR 0.26, p<0.001). Of participants with presumptive acute DEN, 40 (56.3) had no evidence of an acute co-infection. Among 700 samples tested by PCR, all were negative for DEN and flavivirus. Although we were unable to confirm cases by PCR, serological evidence of infection suggests that DEN or a closely related flavivirus is present in Tanzania. Further research is warranted to identify which flaviviruses are circulating in northern Tanzania, including use of virus isolation techniques.

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ROLE OF FUSION LOOP IN ATTACHMENT OF FLAVIVIRUSES TO HUMAN RED BLOOD CELLS

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The flaviviruses West Nile virus (WNV) and Dengue virus (DENV) are a significant public health burden, infecting millions of people worldwide annually. Both DENV and WNV have been found to attach to human red blood cells (hRBCs) during natural infection without losing infectivity, but the significance of this attachment has not been elucidated. Hemagglutination (HA) experiments show that hRBCs agglutinate WNV at a peak pH of 6.2, with HA decreasing at higher and lower pHs. One possible explanation for lack of HA at clinical pH is the involvement of the fusion loop (FL) of the E protein in hRBC binding. That possibility could be verified by assaying HA with non-infectious immature viral particles, in which the prM protein remains uncleaved by furin, blocking exposure of the FL. With that in mind, we investigated the mechanism of binding of flaviviruses to hRBC and the potential involvement of the FL. We used prototype strains for each of the 4 DENV serotypes and a chimeric virus expressing WNV structural proteins with DENV-4 vaccine strain nonstructural proteins (WNV/DENV-4Δ30), grown with or without NH_4Cl to generate wild-type (WT) or predominantly immature virions, respectively. WT and NH_4Cl-treated stocks were characterized by western blot to detect prM in immature particles, focus-forming assay to quantify infectious virus, and qRT-PCR to quantify total viral RNA from mature and immature particles. The ability of WT and NH_4Cl-treated viruses to agglutinate hRBC was determined in comparison with the concentrations of infectious virus and total viral RNA present in each stock. WNV/DENV-4Δ30 and DENVs 2, 3, and 4 had HA titers proportional to the amount of infectious virus but not to total viral RNA, suggesting that immature virus does not agglutinate hRBCs. NH_4Cl-treatment of DENV1 did not alter the proportion of infectious virus compared to total viral RNA, and thus no conclusions could be drawn. Further studies are underway to quantify binding of wild-type and immature virions to RBCs and further clarify the role of the FL in binding.

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NOVEL VIRUS INACTIVATION PLATFORMS FOR A PURIFIED INACTIVATED DENGUE VIRUS VACCINE CANDIDATE

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A number of vaccine technologies are being explored in the development of a tetravalent dengue vaccine. Inactivated viruses offer a simple and cost effective platform for dengue vaccines. Formalin inactivation could lead to cross linking of surface antigens, thus compromising immunogenicity of the product. We have used photo-inactivation of viruses with two novel reagents that inactivate viruses without perturbing the surface antigenic proteins and compared these with formalin inactivated dengue-2 virus. 1,5-iodonaphthylazide (INA) sequesters exclusively into the lipid bilayer of biologic systems and reacts with membrane domains of proteins when exposed to UV radiation. INA has been successfully used in the inactivation of HIV, VEE and other viruses. 4 -aminomethyltrioxsalen (AMT) is a psoralen that inactivates viruses by reacting with the nucleic acid genome. AMT is a low toxicity drug that is already used in cancer chemotherapy and photo treatment of certain skin disorders. Formalin and INA inactivation of dengue-2 led to substantial (30-50 %) loss of binding to 5 different dengue-2 specific monoclonal antibodies whereas binding of antibodies to AMT inactivated virus was comparable to that of un-inactivated virus. Immunogenicity of various inactivated dengue-2 viruses is being tested in a murine model. After a single inoculation with alum adjuvant, all vaccines elicited antibodies as measured by ELISA. These data and virus neutralizing antibody titers after boosting will be discussed.

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MODELS FOR COMBINED NEUTRALIZATION AND ADE OF DENGUE VIRUS BY TWO MONOCLONAL ANTIBODIES

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Most current research on dengue virus (DENV) neutralization uses either polyclonal serum or single monoclonal antibodies (MAbs). Here we describe studies with defined mixtures of MAbs to quantitatively study

the outcome when more that one type of antibody binds to the DENV particle. For these studies we used a panel of 10 well characterized MAbs representing different IgG subclasses, antigen specificities (E, EDIII, prM), serotype cross reactivity patterns (serotype specific or cross reactive), neutralization potency (strong, moderate, weak), and mechanisms of neutralization (pre versus post attachment neutralization). Our data demonstrate that different MAbs function independently, when present in a mixture. For example, we have not observed any synergistic effects when mixtures of neutralizing antibodies were incubated with DENV. Similarly the ability of antibodies to enhance infection of Fc receptor bearing cells was also strictly additive. Finally we observed that neutralization was dominant over enhancement when pairs of neutralizing and enhancing antibodies co-incubated. Based on these results we will present empirical, mathematical models that predict the neutralization and enhancement properties of antibody mixtures.

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LYMPHATIC FILARIASIS AFTER SELECTIVE TREATMENT IN CENTRAL VISAYAS, PHILIPPINES

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Lymphatic filariasis (LF) is endemic in southern half of the Philippines. This study analyzed the incidence rate of LF in Central Visayas, Philippines (2001-2009) recorded at the Department of Health after selective treatment (albendazole and diethylcarbamazine citrate, DEC, on the first day and by 11-day dose of DEC at 6 mg/kg/day) was given in 2001 or 2002. Incidence rates of LF differed (P< 0.05) among the four provinces in the region for the past nine years. Negros Oriental had the highest incidence rate (0.095 cases/1,000 population), followed by Siquijor (0.074 cases/1,000 population), Bohol (0.0043 cases/1,000 population) and Cebu (0.0006 cases/1,000 population). Overall, LF cases have decreased after selective treatment in Central Visayas, however, slight transmission continued in Negros Oriental. The highest incidence rates of the disease in Negros Oriental were found in Sta. Catalina, Mabinay and Siaton, in that order of rank. LF cases were found in four municipalities in Siquijor (Enrique Villanueva, Larena, Lazi, and Maria), three in Bohol (Dimiao, Loon, and Talibon), and only two in Cebu (Liloan and Toledo).

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BARRIERS TO MASS DRUG ADMINISTRATION IN NORTHWESTERN ARGENTINA: IMPACT OF MIGRATION AND REGIONAL WORK PATTERNS

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Soil transmitted helminthiases (STHs) are among the most prevalent neglected tropical diseases, with the highest burden occurring in impoverished populations. Control of STHs is based on periodic targeted or universal deworming, health education and sanitation. In Orán, located in northwestern Argentina on the Bolivian border, nearly half of the population has unmet basic needs. The sugar cane industry is one of the major employers and the seasonal nature of the work results in significant fluctuations in population numbers. In this study we evaluated the first round of a pilot community-wide mass drug administration (MDA) with ivermectin-albendazole in single doses in a rural village, highly endemic for S.stercoralis and other STHs. Through a cross- sectional survey we assessed drug coverage and causes of non-compliance. Sociodemographic data was extracted from the quarterly census done by the local health services. In the survey conducted in September 2010, the total village population was 618. The first round of MDA, which utilized the Primary Health Care System´s network, was done in December on a house by house basis. It took 3 days to complete the drug distribution. By that time, the sugar cane harvest had been completed and 163 persons had migrated out of the community. Of the remaining 455 persons, 12 met ≥ 1 exclusion criteria, 2 refused treatment, and 120 were missed despite repeated household visits. We were able to treat 321 persons, 74% of the available and eligible population. Of missed person, most were men (80%) of working age (median age: 24 years, IQR 16-41). The analysis of the quarterly censuses confirmed the cyclic variations with the total population increasing by over 30% during the harvest season. Maximum drug coverage is essential in order to have an impact on STH morbidity. In our study we identified 2 major barriers: migration and the local work schedule, which affect preferentially the adult male population. Efforts must be made to determine the best strategies for treating this difficult to-reach population.

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FACTORS ASSOCIATED WITH COMPLIANCE WITH MASS DRUG ADMINISTRATION FOR LYMPHATIC FILARIASIS ELIMINATION IN KENYA: DESCRIPTIVE STUDY RESULTS

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Annual Mass Drug Administration (MDA) to at least 65%-80% of population at risk is necessary for Lymphatic Filariasis (LF) elimination. In Kenya, MDA based on diethylcarbamazine and albendazole, has been implemented thrice in Kwale and Malindi districts. To identify factors influencing compliance with MDA, a retrospective cross-sectional study was conducted in the two districts after 2008 MDA. In Kwale, Tsimba Location was selected for high and Gadini for low coverage while in Malindi, Goshi Location represented high and Gongoni low coverage. Using systematic sampling, nine villages were selected from the four locations. Quantitative data was collected from 965 systematically selected household heads and analyzed using SPSS version 15. For qualitative data, which was analyzed manually according core themes of the study, eighty opinion leaders and eighty LF patients with clinical signs were purposively selected and interviewed and sixteen FGDs conducted with adult and youth male and female groups. Compliance among Christians was higher compared to Muslims ($P< 0.001$). Age, sex and marital status did not influence compliance with treatment ($P> 0.05$). There was a significant difference in compliance with treatment among community members with high income levels and those with low income levels ($P< 0.05$). Compliance was higher among community members who had knowledge of signs, cause of LF and considered themselves to be at risk of LF infection compared to those who did not ($P< 0.001$). Compliance was higher among community members who received information that the drugs were given to treat and control LF than those who did not ($P< 0.001$). There is need for investment in reaching out to groups often missed during MDAs. Different strategies have to be devised to reach specific religious groupings and high income earners. All groups targeted for treatment should be educated about the disease and correct information on MDA relayed to them.

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INNOVATIVE WAYS TO CONDUCT COVERAGE SURVEYS IN MALAWI AND MALI

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Mass drug administrations (MDAs) are used to dispense drugs to populations for preventive chemotherapy for five neglected tropical diseases (NTD). Monitoring of drug coverage is crucial for ensuring that

the goals for control and elimination of NTDs are met and is typically done by monitoring reported coverage. WHO recommends periodic validation using household surveys. We sought to investigate alternative survey methods in an effort to find a quick and resource efficient approach that would provide an estimation of drug coverage. Three alternative survey methods were conducted and compared to the WHO recommended 30-cluster method in one district in Malawi and Mali after a MDA for lymphatic filariasis and soil-transmitted helminths had taken place. For the headman method, a village leader, in 30 villages selected by proportional to estimated size (PPES), was asked to designate a person to survey 10 households. For the religious leader method, a leader of a randomly selected religious establishment, in 30 villages selected by PPES, was asked to designate a person to survey 10 households. For the market method, sub-district markets representing geographic coverage of the district were selected. In each chosen market, a convenience sample of 60 people were surveyed. In Malawi, drug coverage for the 30-cluster, market, headman, and religious methods were 66.8% (95% confidence interval [CI]: 60.3%-73.4%), 74.3% (CI: 71.1%-77.4%), 76.3% (CI: 69.6%-83.0%), 77.8% (CI: 72.5%-83.1%), respectively. In Mali, coverage results were 62.6% (CI: 54.4%-70.7%), 56.1% (CI: 48.8%-63.4%), 74.8% (CI: 65.9%-83.8%), and 83.2% (95% CI: 75.8%-90.6%), respectively. All methods were logistically feasible and accepted by survey participants. Technical errors, such as checking multiple answers to a question or not completely filling out the survey, were noted more often for the headman and religious methods. The market, headman and religious surveys required less resources to complete compared to the 30-cluster survey. The market survey method yielded similar results when compared to the 30-cluster survey. Ways to improve the accuracy of the headman and religious surveys have been identified and will be further tested.

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SCHISTOSOMIASIS AND SOIL-TRANSMITTED HELMINTHIASIS CONTROL IN CAMEROON: PROGRESS MADE, CHALLENGES AND WAYS FORWARD

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Recent years have witnessed an increased interest in the control of schistosomiasis, soil-transmitted helminthiasis (STH) and other so-called neglected tropical diseases (NTD). Taking advantage of this new impetus, Cameroon officially launched its national programme for the control of schistosomiasis and STH in 2004. Starting with very limited budget and no external financial support, the control programme gradually mobilized national and international partners, through intense and multifaceted actions, including advocacy and a number of key achievements. In 2005, Cameroon was selected as the start-up country of Johnson & Johnson's mebendazole donation program because of government leadership and commitment to eliminating infections as a major public health problem. This support enabled a rapid scaling-up of activities to encompass all ten regions in 2007. In its efforts to control these diseases, the government of Cameroon adopted an inter-sector collaboration for the implementation of regular school-based deworming activities. In 2009, the Ministry of Health, the Ministry of Education, and the Union of United Councils and Cities signed an innovative tripartite agreement to capitalize their resources. Furthermore, the NTD control in Cameroon is supported by the USAID/RTI/HKI grant since 2010. Through all this partnership, nearly 6 million children are dewormed annually. More than 75 000 teachers, 14 000 headmasters and health personnel were trained. In 2010 and 2011, mapping was achieved in 7 of the 10 regions of Cameroon to update the distribution of schistosomiasis and STH, and to monitor the programme impact. The results showed a significant decrease of infections in all regions. Over the past 5 years significant progress was made for the control of these diseases, as a result of a coordinated effort of the Government with national and international partners. However, there remain several challenges, including integration with other NTDs. The presentation highlights the achievements, challenges, and ways forward for the control schistosomiasis and STH.

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COVERAGE SURVEYS FOR NEGLECTED TROPICAL DISEASES: TEN YEARS OF FIELD EXPERIENCE

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Mass drug administration (MDA), involving the distribution of preventative chemotherapy to an entire at-risk population, is one of the public health strategies recommended by the World Health Organization (WHO) for the prevention, control and elimination of some neglected tropical diseases (NTDs). Adequate coverage is vital to achieve NTD program goals. Reported coverage is often the main indicator used to evaluate NTD programs. The WHO and several drug donation programs recommend conducting coverage surveys periodically to validate reported coverage and to collect additional information necessary to guide NTD programs. Over the past decade, the CDC and collaborators have conducted more than 20 post-MDA coverage surveys in 7 countries throughout the Caribbean, Africa, and Asia. The method used in each surveyed district was a two-stage 30 cluster household survey. For the first stage sampling, clusters were selected with probability proportional to estimated size while second stage sampling used the 'improved random walk method' to select households for interviews. The survey questionnaires were designed to gather coverage data and information relevant to improving NTD programs including: demographic data, MDA distribution strategies, availability of safe water and sanitation, school-attendance, systematic non-compliance, as well as knowledge, attitudes and practices (KAP) surrounding NTDs. We adapted the questionnaire to be used for both individual and integrated drug packages following MDA conducted with varying distribution strategies: house-to-house distribution, school-based distribution, and distribution posts. After a 2-day training and field testing of the questionnaire, three or four teams of 2 persons conducted a survey in 5-7 days. Feasibility in the field was confirmed by the fact that several countries conducted subsequent coverage surveys with little to no technical assistance. Due to the sampling frame, data don't have to be weighted, simplifying the data analyses. Average cost calculations are ongoing. The main challenge was finding a reliable data source with population figures from all villages to establish a sampling frame; however, with one exception, solutions were found. Our experience has led us to conclude that coverage surveys are feasible to implement and can be adapted to multiple settings and to serve multiple program needs.

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A GLOBAL ACCESS FRAMEWORK FOR ADVANCING TRANSLATIONAL RESEARCH IN NEGLECTED TROPICAL DISEASES

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Neglected infections of poverty afflict more than one-sixth of the world's most marginalized populations and reemergence in the developed world remains a viable threat. Medication toxicities, multidrug resistance, and drug pricing severely limit treatment. The United States National Institutes of Health funds 40% of neglected tropical diseases research worldwide and has taken landmark steps to address the drug innovation crisis by establishing the National Center for Advancing Translational Sciences (NCATS). Providing an equitable access framework to health-related innovations and information may allow the Center to best accomplish its applied research goals. Establishing a global access licensing framework for all technology transfers at NCATS may lead to enhanced dissemination of the discoveries generated. Appropriate models for technology transfer would ideally encourage the acquisition of patents for research products only when necessary to promote commercialization, would utilize non-exclusive licensing agreements, create streamlined processes for materials transferred, and reserve broad rights for the use of patented and licensed technologies for future research. Lessons learned from the University of

British Columbia, which effectively applied these principles to transfer a new amphotericin B formulation for treatment of visceral leishmaniasis to the private sector, could provide a framework for future licensing agreements. Prompt public access to NCATS and more broadly, NTD-related manuscripts, may facilitate information exchange and enhance R&D in rare and neglected diseases. An effective strategy may be to alter the current NIH Public Access Policy to require all investigators receiving NIH funding, including those within NCATS, to submit publically available electronic versions of manuscripts to the National Library of Medicine's PubMed Central within one month of the official date of publication. In conclusion, the policy frameworks proposed aim to improve access to information and technologies generated at NCATS and may be necessary to truly advance the translational sciences.

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DID AN IVERMECTIN MDA REDUCE ENDEMIC SCABIES AND STRONGYLOIDIASIS IN A REMOTE ABORIGINAL COMMUNITY IN AUSTRALIA?

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Scabies and strongyloidiasis are endemic in many Aboriginal communities in northern Australia and contribute to the high morbidity experienced by Aboriginal and Torres Strait Islander people. Previous studies have indicated that both parasite infections can be treated with oral ivermectin. We hypothesized that an ivermectin mass drug administration (MDA) program would be an effective public health measure to reduce prevalence of both scabies and strongyloidiasis in remote settings in Northern Territory, Australia. The project includes a population census for prevalence and MDA conducted at month 0 and 12, and a cross sectional survey at months 6 and 18 to identify disease acquisition and treatment failures. Scabies was diagnosed clinically and strongyloidiasis by parasitology through faecal microscopy and/or agar plate culture or by serology. Participants were administered ivermectin in a dose of 200µg/kg unless pregnant or their weight was <15kg. Those not eligible for ivermectin received 5% permethrin or 10% crotamiton and 200mg or 400mg albendazole daily for 3 days. A second treatment was given to those with a diagnosis of scabies and/or strongyloidiasis within 2-3 weeks of the first treatment. The project commenced in March 2011 enrolling 1011 (81%) participants from 127 (80%) houses and 7 (78%) surrounding homelands. Scabies prevalence reduced from 4% at month 0 to a point estimate of 1.8% at month 6 and strongyloidiasis (predominantly diagnosed serologically) from 21% to 6% over the same period. At month 6, disease acquisition of scabies was 1% and strongyloidiasis 3%, with treatment failures of 11% and 16% respectively. The second population census and MDA#2 is currently underway and preliminary results will be presented at the meeting. The study is due to be completed later this year but the early indications for the success of a strategy incorporating mass treatment for both endemic parasitic infections using the one medication are encouraging and could have national and global implications for informing public health programs and treatment guidelines.

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MATERNAL AND CHILD HEALTH IN NORTHERN ANGOLA: MALARIA, SCHISTOSOMIASIS, GEOHELMINTHS, ANEMIA AND MALNUTRITION IN A POST-WAR SETTING

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Parasitic diseases are an important cause of morbidity and mortality worldwide. In Angola, malaria, schistosomiasis and soil transmitted helminth (STH) infections are endemic diseases. National prevalence surveys were conducted in 2007 and 2005, but no detailed updated information exists. The aim of this study was to determine the presence of malaria, schistosomiasis (urinary and intestinal), and STH infections among pre-school (<6 years old), school-aged (6-15 year old) children and their mothers or caretakers in rural and peri-urban areas in Northern Angola (Dande Municipality, Bengo Province). Furthermore, prevalence levels of anaemia and malnutrition were also assessed. We conducted a community-based random sampling survey, between May and August 2010, which included 36 of the 69 hamlets within the CISA Project Demographic Surveillance System (DSS) study area. In total, 972 households were included, representing 960 mothers and their 2379 children (≤15 year olds). Malnutrition and anaemia were found at elevated levels and should be considered severe public health problems, with a total of 21.4% of children being underweight, a prevalence of chronic malnutrition of 32.2% and anaemia reaching 56.9% among under fives. Malaria prevalence in children was close to 18%, and varied heavily according to geographical location, with some hamlets reaching levels above 50%. Similarly, prevalence levels of urinary schistosomiasis depended heavily on location, reaching an overall prevalence of 16.6% in school-aged children. Finally, STH infections were common, with a prevalence of 31.6% in school-aged children. Information gathered during this study will augment previous work by government initiatives and will provide concrete prevalence levels and causal factors for these infections, anaemia and malnutrition on a much smaller geographical scale. More work is needed to better target future campaigns, particularly those aimed at diseases with heterogeneous distributions, such as urinary schistosomiasis and malaria.

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HEALTH SERVICES FOR BURULI ULCER CONTROL: LESSONS FROM A FIELD STUDY IN GHANA

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Buruli ulcer (BU), caused by *Mycobacterium ulcerans* infection, is a debilitating disease of the skin and underlying tissue. The first phase of a BU prevention and treatment programme (BUPaT) was initiated from 2005-2008, in the Ga-West and Ga-South municipalities in Ghana to increase access to BU treatment and to improve early case detection and case management. This paper assesses achievements of the BUPaT programme and lessons learnt. It also considers the impact of the programme on broader interests of the health system. A mixed methods approach included patients' records review, review of programme reports, a stakeholder forum, key informant interviews, focus group discussions, clinic visits and observations. Extensive collaboration existed across all levels, (national, municipality, and community), thus strengthening the health system. The programme enhanced capacities of all stakeholders in various aspects of health services delivery and demonstrated the

importance of health education and community-based surveillance to create awareness and encourage early treatment. A patient database was also created using recommended World Health Organisation (WHO) forms which showed that 297 patients were treated from 2005-2008. The proportion of patients requiring only antibiotic treatment, introduced in the course of the programme, was highest in the last year (35.4% in the first, 23.5% in the second and 42.5% in the third year). Early antibiotic treatment prevented recurrences which was consistent with programme aims. In conclusion, to improve early case management of BU, strengthening existing clinics to increase access to antibiotic therapy is critical. Intensifying health education and surveillance would ultimately increase early reporting and treatment for all cases. Further research is needed to explain the role of environmental factors for BU contagion. Programme strategies reported in our study: collaboration among stakeholders, health education, community surveillance and regular antibiotic treatment can be adopted for any BU-endemic area in Ghana.

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REPORT ON THE MARCH 2011 MCGILL-PAHO WORKSHOP ON DEWORMING OF PRESCHOOL CHILDREN IN THE AMERICAS

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Preschool children are one of three population groups at highest risk for soil-transmitted helminth infections. Because deworming coverage in this group is sub-optimal, new strategies for engagement of governments and other partners are needed. With support from the Canadian Institutes of Health Research, a workshop co-sponsored by McGill University and PAHO was held in March 2011 to review the situation on deworming of preschool children in the Americas. A total of 33 participants represented a variety of organizations, including WHO, the Global Network for Neglected Tropical Diseases, NGOs (including faith-based organizations), academia and national governments. Lessons learned from two long-standing and successful deworming programs, those of Mexico and Nicaragua, were highlighted and served as the basis for a discussion of current challenges experienced in other countries. The workshop recommendations addressed issues of political commitment, integration of deworming with other child health programs, national action plans incorporating NTDs, intersectoral coordination and partnerships, advocacy, capacity-strengthening, community participation and social mobilization, innovation in communication strategies, diagnostic tools, drug formulations and other tools, development and dissemination of guidelines among UN agencies and other organizations, research gaps, South-South collaboration, development of reporting systems, optimal delivery strategies, setting coverage goals and scaling-up activities within the PAHO 10-year plan for Comprehensive Child Health. It is expected that the momentum generated by this workshop, in addition to massive recent donations of deworming drugs, will accelerate national action plans and inform WHO's new Strategic Plan for the Control of Soil-transmitted Helminths for the next decade.

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BIOTECHNOLOGY COMPANIES AND EMERGING MARKET DEVELOPERS PARTICIPATE SIGNIFICANTLY IN R&D FOR NEGLECTED DISEASES

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Understanding the full spectrum of organizations participating in research and development (R&D) for neglected diseases is essential to inform the design of new programs and initiatives to fill gaps in the neglected disease R&D pipeline. In 2011, BIO Ventures for Global Health (BVGH) published an expanded edition of its Global Health Primer, a tool that compiles, tracks, and analyzes the pipeline for drugs, vaccines, and diagnostics in development for neglected diseases. Further analysis of drugs and vaccines in development for 17 neglected diseases was performed using the Global Health Primer dataset and identified 313 products in development by 288 distinct organizations. Organizations identified included academic/research institutions, government agencies, product development partnerships (PDPs), biotechnology companies, and pharmaceutical companies from 42 countries. Two groups of developers identified in this analysis are of particular interest. First, biotechnology companies currently participate in the development of 120 drugs or vaccines for 14 neglected diseases, accounting for 27% of the number of distinct organizations participating in product development. Beyond neglected diseases, the biotechnology sector has played an increasingly important role in the development of innovative solutions to human health challenges. Therefore, increasing participation by this sector represents a key opportunity to increase innovation in the neglected disease R&D pipeline. Second, developers from emerging market countries, focused here on Brazil, China, India, and South Africa, are participating in the development of 42 drugs or vaccines for 13 neglected diseases and represent 15% of the distinct organizations participating in product development. As the economies of emerging market countries grow, they are likely to increase their capacity for scientific research, novel product development, and inexpensive product manufacturing, thus increasing their potential to contribute to neglected disease R&D pipelines. The data presented here will inform future efforts to promote partnering, policy, and financial support mechanisms to engage product developers and address unmet needs in the neglected disease R&D pipeline.

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POST-PREVENTIVE CHEMOTHERAPY COVERAGE SURVEY IN SIERRA LEONE: NATIONAL VALIDATION OF REPORTED DRUG DISTRIBUTION COVERAGE DATA FOR NEGLECTED TROPICAL DISEASE CONTROL

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From October 2008 through February 2009, ivermectin and albendazole were distributed to 3.2 million Sierra Leoneans in 13 districts as preventive chemotherapy (PCT) for lymphatic filariasis, onchocerciasis, and soil transmitted helminthes. Although supervision by national program staff during PCT was routinely conducted, a post-PCT coverage validation survey was conducted in April 2009 so that reported coverage rates could be validated, detailed information could be collected on gender- and age-specific coverage by district, reasons why people chose not to take the drug could be compiled, and the current strategies assessed. At the national level and in eight districts, reported coverage rates fell outside of the surveyed coverage confidence intervals for ivermectin and/or albendazole. The largest differences between the two coverage rates were in the districts of Kono and Moyamba, the two districts with the greatest variation in population changes due to post-war migration, with increases that are not reflected in the national census projections. In the Rural Western Area health district, the large confidence intervals suggested a need to validate coverage through alternative means. Gender- and age- specific survey data were assessed: in ten districts, there were no significant differences in coverage between males and females, while females were significantly less likely to be treated compared to males in three districts ($p < 0.05$). Of the age categories examined, those 15-29 years old were the least likely to take both drugs compared to ages 5-14 and those greater than 30 years. The most common reasons

given for not receiving treatment were being underage, absent at the time of distribution, and that the drug distributor did not visit the house. Recommendations based on survey results will be used in subsequent years to strengthen the PCT strategies through increased field supervision, heightened monitoring and evaluation, improved drug availability, more robust social mobilization campaigns, and an improved method to validate coverage data.

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BREAKING INTELLECTUAL PROPERTY (IP) BARRIERS TO ACCELERATE DRUG RESEARCH AND DEVELOPMENT (R&D) FOR NEGLECTED TROPICAL DISEASES

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The Pool for Open Innovation against Neglected Tropical Diseases ("the Pool"), administered by BIO Ventures for Global Health, motivates innovative and efficient drug discovery and development by opening access to intellectual property (IP) or know-how in neglected tropical disease (NTD) research. Intellectual property concerns have been at the heart of access to medicines. Intellectual property includes patents_which give the owner a period of time to exclusively market a new product, and know-how the accumulated experience that companies gain over time through working on new drugs. The Pool makes thousands of patents and associated know-how accessible to qualified researchers working on research and development (R&D) on drugs for NTDs, allowing these researchers to take advantage of hundreds of millions of dollars of value accumulated in the IP of companies and universities. The project engages novel mechanisms, such as profiling specific patents with potential application to new drug discovery and development for NTDs, to make the patents more accessible to researchers and highlight new project opportunities focused on drug discovery and development. For example, GlaxoSmithKline (GSK) has contributed a family of patents covering small molecules with antibacterial activity. GSK has biological data relevant to tuberculosis and malaria for compounds based on this chemical scaffold that can serve as a starting point for follow on work in tuberculosis and malaria or novel projects for leprosy and Buruli ulcer. Ultimately, by opening access to their IP, contributing organizations offer an opportunity to gain invaluable 'know-how' that can advance existing research efforts. This poster will provide a brief overview of the project structure and core principles, as well as demonstrate examples of how researchers can engage with the Pool to accelerate the pace of R&D for NTDs.

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AZITHROMYCIN DISTRIBUTION USING COMMUNITY VOLUNTEERS: COSTS OF DISTRIBUTION IN SEVEN DISTRICTS IN PLATEAU AND NASARAWA STATES, NIGERIA

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Mass treatment with azithromycin is a key component of the WHO SAFE strategy to eliminate blinding trachoma. In Nigeria, the first ever mass administration of donated azithromycin to combat blinding trachoma (donated by Pfizer for this purpose) took place in 2010. As an alternative strategy to current trachoma control program practice, MDA in Plateau and Nasarawa states was carried out by community volunteers, with support from the Ministry of Health and The Carter Center. A total of 769,517 treatments in 7 local government areas (districts) of either azithromycin tablets, pediatric oral suspension, or ophthalmic tetracycline ointment were distributed representing an estimated 77.7% coverage of the total population. The Ministry of Health provided personnel but no direct costs; since The Carter Center provided all direct funding, all costs could be monitored through financial records. Costs were assessed according to input (per diem, transport, and materials and supplies) as well as activity (advocacy, training, distribution, and supervision). A total of $47,243 was spent, $20,762 (43.9%) of which was used for clearing and shipping of the drug. The remaining $26,481 accounted for all distribution costs. Personnel costs (per diems) was the largest input at $14,684 (55.5%) while training of MOH supervisors and community volunteers accounted for greatest activity cost at $10,704 (40.4%). The total and mean cost per treatment was $0.04 (range $0.02 to $0.05), not including clearing and shipping costs. Not included in this analysis were salaries, overhead costs for The Carter Center or Ministry of Health, or drug costs (except tetracycline eye ointment). Some economies of scale were seen in larger local government areas where per person treatment costs reduced compared to smaller local governments.

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DETERMINATION OF THE SENSITIVITY AND SPECIFICITY OF THREE SERODIAGNOSTIC ASSAYS FOR CHAGAS DISEASE BY LATENT CLASS ANALYSIS

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Chagas disease, caused by the hemoflagellate *Trypanosoma cruzi*, is an increasing public health concern in the Unites States because of the estimated 300,000 infected residents, the majority of who came to the country from endemic areas. During the extended chronic stage of this disease, the parasitemia is very low and difficult to detect, therefore serological assays assume primacy for diagnosis. There is no gold standard for the serodiagnosis of Chagas disease, thus the WHO recommends that diagnosis is based on the concordant results of 2 different serologic tests. In the absence of a gold standard, diagnosis of disease is imperfect. We used Latent Class Analysis (LCA) to determine the sensitivity and specificity of 3 serodiagnostic assays for Chagas disease. LCA statistically models the results from the different tests to the underlying latent classes (positive or negative). The analysis yields probabilities for each combination of results from which the sensitivity and specificity of the each assay can be estimated. To generate the data for LCA, we tested a serum bank with 3 assays: the Trypomastigote Excreted Secreted Antigen Immunoblot (TESA IB), the CDC Chagas immunofluorescence assay (IFA) and the commercial Chagatest *ELISA recombinante v.3.0* (Wiener Laboratorios, Argentina). The serum bank (n = 605) comprised sera submitted to CDC for routine diagnosis of Chagas disease (n = 479), plus 126 true negative specificity controls that were positive for diseases which can cause cross reactivity in Chagas serology and came from areas where Chagas disease is absent or transmission is very rare. We performed LCA on the entire set (n = 605). All 3 assays returned sensitivity and specificity values >94%. We also estimated the specificity of each assay by standard 2 x 2 tables using data from the true negatives (n = 126). These specificity results were: TESA IB 99.2%, IFA 93.7%, Chagatest ELISA 86.5%. These data provide an independent estimate of the performance of these assays and support their use for serodiagnosis.

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IN THE SEARCH FOR MARKERS OF CHEMO-RESISTANCE IN AMERICAN TEGUMENTARY LEISHMANIASIS (ATL)

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Therapeutic failure in leishmaniasis is a common problem in endemic areas. This may occur due to altered drug pharmacokinetics, re-infection, or immunologic compromise of the host. However, in many cases it may be partly due to parasite drug resistance. No reliable markers of chemo-resistance against leishmanicidal drugs have been described until yet, and the only reliable method for monitoring resistance of individual isolates is the *in vitro* amastigote-macrophage model, as reported

previously. It is thus necessary to uncover cellular and molecular indicators to be used systematically to identify the drug-resistant phenotype of the infecting parasites. Herein we analyze in parasites isolated from three patients suffering ATL and lack of response to antimonials their capacity to accumulate calcein, the rate of glucose uptake and the membrane potential, and compared the results with those obtained from references strains belonging to *Leishmania. braziliensis L. mexicana* and *L. amazonensis*. Our results suggest that some of the isolates a) have an increased expression of ABC transporters; b) accumulate glucose at a lower rate and c) have a less polarized membrane potential compared to the reference parasites. Additionally they suggest that some of these isolates express different sensitivity of the membrane potential to classic inhibitors of the mitochondrial function. Altogether these results indicate that in parasites isolated from ATL patients suffering chemotherapeutic failure there could be physiological changes that might serve as markers of chemo-resistance and be helpful for designing strategies to circumvent *Leishmania* drug-resistance and successfully treating leishmaniasis. If this conclusion holds true particularly in isolates obtained from patients, its prognostic value treatment outcome might be extremely useful.

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ANALOGUES OF FENARIMOL AS NOVEL COMPOUNDS FOR THE TREATMENT OF CHAGAS DISEASE

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A collaborative drug discovery consortium established by not-for-profit, drug research and development organization Drugs for Neglected Diseases *initiative* (DND*i*) has identified and developed novel compound series active against intracellular protozoan parasite *Trypanosoma cruzi* the causative agent of Chagas disease. Compounds are derived from the fungicide fenarimol reported to inhibit ergosterol biosynthesis by binding to fungi CYP 51. Fenarimol derivatives are able to suppress bloodstream parasitemia to virtually undetectable levels after once-a-day oral dosing in a mouse model of chronic *T. cruzi* infection. Compounds are non-cytotoxic and chemically tractable allowing rapid optimization of target biological activity and drug characteristics. Chemical and biological studies undertaken in the development of this series of compounds towards the goal of delivering new drug candidates for Chagas Disease will be presented.

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A MEMBER OF RAS ONCOGENE FAMILY, RAP1A SIGNALING, MEDIATES ANTILEISHMANIAL ACTIVITY OF MONASTROL

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Microarray experiments were conducted on Affymetrix GeneChip® HG-U133 Plus 2.0 array to determine the genes that encode proteins related to pathological alterations of cell signaling pathways in intracellular *Leishmania* amastigotes in response to the oral antileishmanial agent, monastrol. Monastrol, the investigational compound, with antileishmanial activity targeting pteridine reductase (PTR1) in *Leishmania* parasites, induced unprenylated Rap1A when exposed to this anticancer drug at IC_{50} of 10 μM. Monastrol is known to cause mitotic arrest in cancer cells, inhibited Rap1A prenylation (geranylgeranylation) in intracellular *Leishmania* which results in blockade at the G1 phase of the cell cycle. Regulators (unprenylation) of cell signaling pathways can be exploited in *Leishmania* parasites as novel therapeutic tools. We propose the development of antiparastic drugs to 'piggyback' on the development of inhibitors for cancer research targeting farnesyltransferase and geranylgeranyltransferase.

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MOLECULAR DIAGNOSIS OF LEISHMANIASIS AT THE COMPLEX AND SPECIES LEVEL IS IMPORTANT FOR CLINICAL MANAGEMENT

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The intracellular parasite *Leishmania* causes a wide spectrum of human disease, ranging from self-healing cutaneous leshimaniasis to fatal visceral leishmaniasis. *Leishmania* is a digenetic obligate intracellular protozoan parasite. Management depends on the clinical syndrome which is a function of the species complex. Drug resistance has also been associated with certain species. Culture or stain-based methods do not distinguish complex or species of *Leishmania*. We report a real-time PCR (RT-PCR) assay for laboratory diagnosis of Leishmaniasis by the detection of *Leishmania* complexes (L. Viannia, *L. mexicana*, *L. donovani/infantum, L. major, L. tropica*) directly from clinical samples. To highlight the utility of molecular detection and complex idenfictcation two clinical cases of cutaneous leishmaniasis are presented. Case 1 was a 31 year-old-man born in Syria who immigrated to Canada one year prior to presentation. He had an 18 month history of numerous cutaneous lesions on his forearms bilaterally which could be interpreted as disseminated disease consistent with a more virulent species. The possibility of visceral disease prompted a more aggressive approach to management in this individual with sodium antimony gluconate 1900 mg IV daily for 20 days. The RT-PCR performed on skin scraping of the lesion from this case interestingly identified species *L. tropica*. Case 2 was a 49 year-old-man born in Canada. He developed a papule on his right arm which later ulcerated four months after he traveled to Surinam for one month. The patient did not receive treatment. The cutaneous leishmaniasis lesion healed spontaneously with some postinflammatory hyperpigmentation. The RT-PCR performed on skin scraping of the lesion from this case however identified *L. (Viannia) panamensis* (Braziliensis complex). We conclude that *Leishmania* complex or species identification is useful in the management of this disease.

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DRUG DISCOVERY ALGORITHM FOR CUTANEOUS LEISHMANIASIS

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The results of our automated, high-throughput screening of potential drugs *in vitro* against promastigotes was recently published and the complimentary exercise against axenic amastigotes will be detailed elsewhere. Because no drug has been specifically developed for cutaneous leishmaniasis, there are no examples of a pre-clinical product evaluation scheme that leads to agents for formal non-clinical and clinical development. We have developed a testing strategy that features a gated, resource sparing model that progresses from high-throughput *in vitro* promastigote and axenic amastigote assays to more clinically relevant, comparable mouse *Leishmania* suppression and *Leishmania* cure models that have undergone internal validation and are reproducible. Our process for advancing compounds from hit to lead will be discussed.

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OPTIMIZING ANALOGS OF TIPIFARNIB AS *TRYPANOSOMA CRUZI* CYP51 INHIBITORS

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Tipifarnib is an oral anti-cancer drug clinical candidate that blocks mammalian protein farnesyltransferase (PFT). In previous work, we reported that tipifarnib is a potent inhibitor of *Trypanosoma cruzi* growth, but acts via inhibition of the parasite's sterol 14α-demethylase (CYP51) enzyme. Since the antitrypanosomal activity is unrelated to inhibition of PFT, new tipifarnib analogs were designed to minimize PFT inhibition and to maximize binding to the *T. cruzi* CYP51. The purpose was to eliminate side effects, such as bone marrow suppression, that are associated with PFT inhibition. The design of analogs was aided by the crystal structures of both mammalian PFT and the *T. cruzi* CYP51. Compared to the parent compound (tipifarnib), the analogs have as much as 10,000-fold higher IC_{50} values on mammalian PFT and >10-fold lower EC_{50} values on *T. cruzi* cultures (in the sub-nanomolar range). The compounds rank amongst the most potent anti-*T. cruzi* compounds that have ever been discovered. The compounds also have potent activity in the mouse model of *T. cruzi* infection, and new *in vivo* data will be presented. By starting with the tipifarnib scaffold, we hope to retain some advantageous pharmacological properties, namely, oral bioavailability with excellent human pharmacokinetics, low inhibitory activity on liver P450 enzymes, and a simple chemical structure with low cost of goods. These intrinsic attributes may have advantages over antifungal CYP51 inhibitors that are also under investigation for Chagas disease.

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ISOLATION OF NOVEL STEROLS WITH ANTI-LEISHMANIAL ACTIVITY FROM THE MAYAN PLANT *PENTALINON ANDRIEUXII* MUELLER-ARGOBIENSIS

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Pentalinon andrieuxii has been used by traditional Mayan healers for topical treatment cutaneous leishmaniasis in the Yucatan Peninsula. Chemical analysis of this plant revealed the presence of a new cholesterol derivative, that we named pentalinonsterol, and a new polyoxygenated pregnane sterol glycoside that we named as pentalinonoside, we also isolated 18 known compounds, that includes 14 sterols, three coumarins, and a triterpene. All these compounds were isolated from an *n*-hexane partition of a methanol extract of the roots of the plant. Structure elucidation of all 20 compounds was accomplished by spectroscopic procedures. Experiments performed *in vitro* revealed that 6 out of those 20 compounds present a strong antileishmanial activity against promastigotes of *Leishmania mexicana* as detected *in vitro* by flow cytometry. Additionally, sterols were active against intracellular amastigotes grown in mouse macrophages. We conclud that six sterols from *P. andrieuxii* present anti-leishmanial activity *in vitro* and as such could be leads for developing new drugs.

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IDENTIFICATION OF ANTILEISHMANIAL BENZOTHIAZOLES BASED ON HITS FROM A HIGH-THROUGHPUT PROMASTIGOTE SCREEN

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A screen of ~200,000 compounds from the PubChem database revealed 93 compounds that possessed IC_{50} values ≤ 1 μM against *L. major* promastigotes, as reported previously. To identify new compounds active against intracellular *Leishmania*, thirty-four of these compounds were selected according to chemical exclusion criteria and availability, purchased from commercial suppliers, and evaluated for *in vitro* activity against intracellular *L. donovani* and *L. amazonensis* parasites. Benzothiazole compounds (PubChem 16196319 and 16196223) related to cyanine dyes exhibited potent activity against intracellular amastigotes, leading to a search for structurally related and commercially available compounds. The cyanine dye thiazole orange (Pubchem 123859) showed exceptional *in vitro* antileishmanial activity, particularly against intracellular *L. donovani* (IC_{50} = 21 ± 12 nM) and low cytotoxicity against Vero cells (IC_{50} = 7800 ± 231 nM). Dithiocarbamates also showed nanomolar *in vitro* antileishmanial activity, as the aldehyde dehydrogenase inhibitor disulfiram possessed *in vitro* potency (IC_{50} = 43 ± 6 nM) which was similar to the reference drug amphotericin B. Several of the most potent compounds have been evaluated for their efficacy in a murine model of visceral leishmaniasis. Thus far, the most promising compounds from *in vivo* studies have been benzothiazoles 123859 and 16196319. When given at a dose of 1 mg/kg i.p. daily for five days, 123859 and 16196319 cause 44% and 42% suppression of liver parasitemia in *L. donovani*-infected BALB/c mice, respectively, compared to the untreated control group. Benzothiazole-containing cyanine dyes are thus potential lead compounds for the discovery of novel antileishmanial agents.

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OPTIMIZATION OF CONGENITAL CHAGAS DISEASE TREATMENT WITH BENZNIDAZOLE

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The main difficulty in the treatment of congenital Chagas disease regards compliance to the treatment: over 40% of treatments are stopped before achievement. We conducted a clinical trial to compare two doses of benznidazole to see whether the simplification and reduction of treatment could induce a better compliance. The study was conducted in 3 hospitals in the city of Santa Cruz in Bolivia. Newborns of all seropositive women for *Trypanosoma* cruzi were investigated for *T. cruzi*. We confirmed the cure for all children by parasitological examination at 1 and 2 months and by serological surveillance using Chagas Stat-Pak®, confirmed by an ELISA using recombinant antigens (Chagatest®, Wiener, Argentina) performed at 1, 2 and 9 months. The comparison of compliance between the two groups was based on both the use of electronic pillboxes (MEMS®, AARDEX, Switzerland) registering each opening of the bottle, and a weekly visit at home to check treatment attendance. We compared the total dose taken with the prescribed dose. Infected newborns were randomly divided into 2 groups: 64 infants received treatment A (5 mg/kg per day in 2 divided doses for 60 days) and 61 infants were treated by treatment B (7.5 mg/kg per day in 1 single dose for 30 days). Taking into account refusals, abandonments and deaths, the numbers of followed infants were respectively 59 and 54 infants. There was no significant difference (P> 0.05) between the groups A and B regarding a) the average number of days without treatment, b) the frequency of days without

treatment, c) the number and importance of treatment-free periods exceeding 3 consecutive days and d) the prescribed dose and the dose taken. Our results showed that compliance was not significantly improved by streamlining the processing or shortening. However, simplification of treatment (once daily instead of two) could allow, at a time, to reduce the doses and maintain the same efficiency.

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IDENTIFICATION OF SERUM BIOMARKERS FOR CHAGAS DISEASE

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The blood borne pathogen *Trypanosoma cruzi*, is the etiological agent of Chagas disease in humans. Following a natural infection some individuals exhibit an acute phase, with parasites present in blood, while 20 to 30% of individuals develop chronic Chagas disease with clinical symptoms starting many years after the initial infection. Most chronically infected individuals show no clinical symptoms and may donate blood, resulting in an increased risk of transfusion transmitted Chagas disease. Parasites are rarely detected in blood in chronically infected individuals. To overcome the difficulty of detecting parasites directly, diagnostic assays detect host anti-*T. cruzi* antibodies as a surrogate marker for infection. However, these assays are not reliable during the initial window period, or to follow cure after drug treatment due to the persistence of parasite specific antibodies. Previous studies have determined that the parasites secrete various antigens in the blood and these have been collectively termed as *T. cruzi* Excreted Secreted Antigens (TESA). We utilized *in-vitro* RNA SELEX methods to develop TESA aptamers (short nucleic acid molecules) with the goal of utilizing them as specific ligands in detection assays. The TESA SELEX was performed using culture supernatants of *T. cruzi* trypomastigote infected NIH-3T3 cells. Biotinylated monoclonal TESA specific aptamers were utilized in a modified enzyme linked assay to detect TESA antigens in *T. cruzi* infected mouse plasma. Aptamer L44 (AptL44) demonstrated a consistent and strong binding to its target in infected mouse plasma during the acute phase. This interaction was specific as a scrambled aptamer did not bind to either infected or uninfected mouse plasma. AptL44 was also able to detect its target in plasma from chronically infected mice, 135 days post infection, where no parasites were detectable in blood by microscopy. Further analysis of the binding properties of AptL44 and the identification and purification of its target are being carried out. This is the first demonstration of an aptamer based assay that detects a parasite biomarker for the diagnosis of Chagas disease.

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DRUG SUSCEPTIBILITY OF *LEISHMANIA* VIANNIA SPECIES IN COLOMBIA

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Treatment failure is frequent and concerning in the management of cutaneous leishmaniasis in South America. Treatment with Glucantime® has been shown to select antimony (Sb^v)-tolerant/resistant parasites and drug resistance to contribute to treatment failure. To determine the susceptibility of *Leishmania* affecting human populations in Colombia to currently used anti-leishmanial drugs, we evaluated *in vitro* susceptibility of 150 clinical strains of *L. braziliensis, L. panamensis* and *L. guyanensis* from endemic regions to Glucantime® and miltefosine. Susceptibility was determined based on reduction of intracellular parasite burden in U-937 macrophages by screening at single drug concentrations and ED50 determination. Sb and miltefosine resistant lines and their wild type strains provided internal standards. Susceptibility to miltefosine and Glucantime® differed among species and by geographic origin. Low susceptibility was defined as < 50% reduction of parasite burden at the screening concentration of 32mgSb^v/ml (based on C_{max} of Sb in plasma) and 16uM for miltefosine (based on toxicity of higher concentrations for U-937 cells). 20-50% of *L. panamensis* and 40-53% *L. braziliensis* strains presented low susceptibility for Sb^v; 14-80% of *L. panamensis* and 58-79% for *L. braziliensis* presented low susceptibility to miltefosine. All *L. guyanensis* strains were highly susceptible to both Sb^v and miltefosine. *Leishmania* from the Orinoco and Amazon River regions were less sensitive to both drugs than strains from other high transmission areas. *L. braziliensis* presented low sensitivity to both drugs more frequently than other *(Viannia)* species. No significant difference in susceptibility to Sb was detected among strain cohorts (N=85) isolated between 1980-1989 and 2000-2009 in the municipality of Tumaco. However a higher proportion of strains from the Rosario river focus presented low susceptibility than strains from the Mira river focus (50% vs 27%, *p=0.032*). These results support both intrinsic and acquired differences in drug susceptibility of *L. (Viannia)* species.

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CYP51 INHIBITORS IN CLINICAL TRIALS FOR THE ETIOLOGICAL TREATMENT OF CHAGAS DISEASE

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Chagas disease, caused by the kinetoplastid protozoon *Trypanosoma cruzi*, remains the highest parasitic disease burden in the American continent and is now spreading to non-endemic countries due to international migrations. Specific chemotherapy of this complex and long-neglected disease remains unsatisfactory due to limited efficacy of currently available drugs (nifurtimox, a 5-nitrofuran and benznidazole, a 2-nitroimidazole), particularly in the prevalent chronic stage, as well as unwanted side effects that can lead to treatment discontinuation. Currently, the most advanced candidates for new specific treatments are a group of third-generation triazole derivatives, originally developed for the treatment of invasive fungal infections, which are potent and selective inhibitors of fungal and protozoal cytochrome P-450-dependent C14α sterol demethylase (CYP51). These compounds have been shown to induce radical parasitological cures in different animal models of acute and chronic Chagas disease, being active *in vivo* against nitrofuran- and nitroimidazole-resistant *T. cruzi* strains, even if the hosts are immunosuppressed. The remarkable *in vivo* antiparasitic activities of these CYP51 inhibitors result from a combination of their potent and selective intrinsic anti-*T. cruzi* activity with special pharmacokinetic. Among this group of compounds, posaconazole (Noxafil®, Merck) and ravuconazole (Eisai), both of which have completed preclinical studies, are currently entering clinical trials for the etiological treatment of chronic Chagas disease. A Phase II clinical trial of the comparative efficacy and safety of posaconazole and benznidazole in chronic Chagas disease patients was started in Vall d'Hebron University Hospital, Barcelona, Spain in October 2010. Also in 2010, Merck announced plans to initiate a Phase II investigational proof-of-concept clinical study to evaluate posaconazole for the treatment of chronic Chagas disease in Argentina and Brazil, with an estimated start date of 2Q 2011. On the other hand, the Drugs for Neglected Diseases initiative (DNDi) announced in 2009 that it had reached an agreement with Eisai for the clinical development of E1224, a water-soluble pro-drug (mono-lysine derivative) of ravuconazole, for the treatment of chronic human Chagas disease in Bolivia; the estimated start date is April 2011.

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INTERACTION MAP OF LYT1 FROM *TRYPANOSOMA CRUZI*

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Trypanosoma cruzi is the etiologic agent of Chagas' disease. This parasite requires infecting the host cell to complete its intracellular cycles, a process in which the involvement of very few molecules of the parasite has been described. LYT1 is a lytic molecule active in acid conditions that, by genetic approach, we demonstrated its participation in the parasite infection and stage-transition processes. This multi- functionality are the result of the production of two LYT1 products obtained by alternative trans-splicing in which the full-length LYT1 protein contains an amino-terminal signal sequence and an internal sequence which directs nuclear localization, whereas the truncated protein lacks the secretion sequence. Therefore, one form of the LYT1 protein is secreted and participates in hemolysis, infectivity and the parasitophorous vacuole escape. The other form is located in the kinetoflagellar and nucleus zone and is involved in the parasite developmental process. This dual/single-gene expression and consequent differential localization and functional switching of protein products, expose these molecules to different microenvironments that could impact on protein folding and interaction with other proteins. Therefore, in this work we performed co-immunoprecipitation and GST pull-down assays followed by MS-MS analysis, to obtain the LYT1 interactome. The co-immunoprecipitation assays demonstrated than LYT1p interacts with at least 14 proteins from 8 to 255 kDa range of molecular weight, which interact with different forces according with crescent salt stringency experiments. In the same way, by GST pull-down assays we observed an interaction with at least 8 proteins from 27 to 100 kDa range of molecular weight, which also showed different interaction forces. The MS-MS analysis allows us to identify proteins that are related with the infection or stage-transition process of *T. cruzi*, in which the participation of LYT1p has been demonstrated.

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DIFFERENTIAL GENE EXPRESSION IN DEVELOPMENT OF *TRYPANOSOMA BRUCEI* DURING SALIVARY GLAND COLONIZATION OF *GLOSSINA MORSITANS MORSITANS*

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African trypanosomes (*Trypanosoma brucei* sspp) are the etiological agent of the fatal human disease, Sleeping Sickness, in sub-Saharan Africa. This parasite is transmitted to the vertebrate host by the bite an infected tsetse fly (*Glossina* spp.). When the fly feeds on an infected host, trypanosomes differentiate to the procyclic form in the midgut (MG). Parasites migrate to the proventriculus (PV) and then to salivary glands (SG), where epimastigotes attach to the epithelium, and differentiate ultimately becoming the mammalian-infective metacyclics. SG invasion occurs in only a subset of flies that carry midgut infections. In non-permissive flies, the infection is halted in the PV. The molecular aspects that govern SG invasion are currently unknown. In this work, from an Illumina transcriptome data set, we have analyzed the differential regulation of four putative proteins sharing type II phosphatidic acid phosphatase (PAPs) motifs. In eukaryotic cells, PAP activity has a central role in phospholipids and triacylglycerol synthesis through its product diacylglycerol, and also generates and/or degrades lipid-signalling molecules related to phosphatidate. Three of the four proteins are predicted to be transmembrane proteins that cross the lipid bilayer six times. Structural homology comparison suggests that the catalytic site of these enzymes is exposed to the extracellular mileu. cDNAs from infected tissues were normalized to trypanosome alpha-tubulin using RT-PCR. Normalized cDNAs were tested with gene specific primers for the four genes. Further, PV cDNAs (prepared as above) from both SG permissive and non-permissive flies were similarly analyzed. Three of four genes were found to be highly expressed only in infected SG. Genes expressed highly in the SG were also found to be expressed at a higher level in the PV from SG permissive flies. We hypothesize these enzymes could be involved in cell signaling processes regulating SG invasion by *T. brucei* and/ or *T. brucei* differentiation in permissive flies. Characterization of these proteins could increase our understanding of SG invasion processes by *T. brucei*.

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DIFFERENCES IN THE *IN SITU* INFLAMMATORY REACTION OF THE AMERICAN TEGUMENTARY LEISHMANIASIS AND SPOROTRICHOSIS AS AN EXAMPLE OF THE SKIN IMMUNE SYSTEM RESPONSE PATTERNS

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The skin is an important immune surveillance organ that is target by many infectious agents. The clinical presentation of skin infectious diseases can be influenced by the interaction between the skin immune system (SIS) and intracellular or extracellular pathogens, such as *Leishmania* spp and Sporothrix schenckii, respectively. As concequence, American tegumentary leishmaniasis (ATL) and sporotrichosis (SP) could be influenced by the pathogen-skin immune system (SIS) interaction. To better clarify the underlying mechanisms of skin inflammation in the presence of different pathogens, we used immunohistochemistry to analyze 3 groups of patients with lymphocutaneous (LC) and fixed (F) forms of sporotrichosis and cutaneous form of ATL. ATL lesions had a significantly higher percentage of $CD3^+$ cells than LC ($p= 0.012$) and F ($p= 0.009$), $CD8^+$ cells ($p= 0.001$ and $p= 0.002$, respectively), macrophages ($p= 0.003$ and $p= 0.025$), $FasL^+$ cells ($p= 0.001$ and $p= 0.003$) and NOS2 ($p= 0.007$ and $p= 0.0001$). In contrast, LC lesions had a significantly higher percentage of dendritic cells ($p= 0.026$), neutrophils ($p= 0.009$) and $CD22^+$ cells ($p= 0.024$), than ATL lesions. The clinical presentation of ATL and sporotrichosis could be due to a combination of factors from the host SIS and etiological agent. In addition, the results also indicated a different profile of the *in situ* immune response when ATL, LC-SP and F-SP are compared, suggesting that the SIS is a complex, adaptable system that is capable of different responses to intracellular or extracellular pathogens.

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DRUG LEADS TARGETING *TRYPANOSOMA CRUZI* CYP51 IDENTIFIED BY HIGH-THROUGHPUT SMALL MOLECULE SCREENING

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Chagas Disease, a WHO- and NIH-designated neglected tropical disease, is endemic in Latin America and an emerging infection in the US and Spain as a result of population movements. Although a major cause of morbidity and mortality due to heart failure, as well as exacting a heavy economic toll in affected regions, Chagas Disease attracts little attention from the pharmaceutical industry because of adverse economic incentives. Discovery and development of new routes to chemotherapy for Chagas Disease is a clear priority. To maintain membrane integrity, *Trypanosoma cruzi*, the etiological agent of Chagas Disease requires endogenously synthesized episterol and fecosterol, membrane building blocks that cannot be entirely substituted by cholesterol scavenged from the host. The similarity between the sterol requirements of pathogenic fungi and *T. cruzi* validated the strategy of repurposing anti-fungal drugs inhibitors of CYP51 to treat Chagas Disease. To supply the therapeutic pipeline with novel

anti-Chagasic drug candidates, we exploited a complementary approach that relies on directly probing the *T. cruzi* CYP51 active site with synthetic small molecules to select chemotypes with high binding affinities to the target. Our approach incorporates screening technologies against both the target enzyme and the parasites, x-ray crystallography and computation. This strategy allows the unique binding features of positive hits to be elucidated, with the ultimate goal of utilizing them in the subsequent hit-to-lead optimization. This approach has enabled us to identify novel scaffolds which show significant diversification from previously identified anti-fungal drugs. One potent *T. cruzi* inhibitor with drug-like properties is currently being optimized.

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GENE EXPRESSION PROFILES OF HUMAN MACROPHAGES INFECTED WITH *LEISHMANIA BRAZILIENSIS IN VITRO*

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The protozoan parasite *Leishmania braziliensis* has a high degree of intra-species genetic and phenotypic polymorphism, which is accompanied by a spectrum of clinical presentations in the infected human host, including: localized cutaneous leishmaniasis (CL), mucosal leishmaniasis (ML) and the more recently described disseminated leishmaniasis (DL). Our hypotheses are (1) that these parasites interfere with the gene expression of infected cells in a manner that is beneficial to their infectivity, and (2) that strains of *L. braziliensis* drawn from patients with either CL, ML or DL lead to different gene expression profiles in the infected macrophages. Employing DNA micro-array we compared the global gene expression profiles in human monocyte derived macrophages (MDM), obtained from healthy donors and infected in parallel with one *L. braziliensis* isolated from a CL, one from a ML and one from a DL case of the same endemic region in Northeastern Brazil. We also assessed how infected MDM compared with non-infected cells. Overall, *L. brazilienis* caused the repression of the majority of the genes that presented significant changes of their expression levels in infected MDM as compared to non-infected cells. In this respect, genes belonging to the stimulus transmission, apoptosis and reactive oxygen production pathways were the most affected. Interestingly, genes for proteins involved in stress protection were up-regulated. Among the three isolates tested, the two drawn from metastatic disease cases (i.e. ML and DL) induced more similar gene expression patterns in the MDM. The findings suggest that these parasites may increase their chance of survival by down regulating host cell genes during the infection process, and that strains associated with different forms of disease elicit somewhat diverse behaviors in host cells, which may be related to the different clinical outcomes of the disease.

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THE COMPLEX ROLE OF PROGRAMMED DEATH-1 (PD-1) IN CHRONIC ZOONOTIC CANINE VISCERAL LEISHMANIASIS

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The ability of the immune system to effectively respond to *Leishmania infantum* infection, the causative agent of visceral leishmaniasis (VL), is dependent upon a classic Th1 response including antigen-specific proliferation of CD4+ T cells. Functional exhaustion of T lymphocytes has not previously been identified during VL. Treatment of clinical VL is complicated by an attenuated immune response at the time of therapy, resulting in incomplete parasite clearance and possible recrudescence. Zoonotic canine VL serves as a pertinent and useful model to better understand cellular mechanisms which alter clinical disease. Our previously published research suggested the presence of CD4+ T-cell exhaustion in poly-symptomatic dogs chronically infected with *L. infantum,* as evidenced by their significantly reduced $CD4^+$ T cell proliferation in response to specific antigen and significant production of IL-10 after *ex vivo* antigen stimulation. Our hypothesis was that peripheral blood mononuclear cells (PBMC) from dogs diagnosed with *L. infantum* via serology and qRT-PCR would have increased expression of the surface receptor PD-1. PBMC from dogs in three different symptomatic states were evaluated for responsiveness to *L. infantum* antigen, surface expression of PD-1, and production of IL-10 and IFN-γ ex vivo. CD4+ T cells from dogs with immune exhaustion had significantly elevated PD-1 expression compared to naive and infected immune-responsive dogs. Dogs with elevated PD-1 and poly-symptomatic clinical disease demonstrated decreased IFNγ producing CD4+ cells and increased IL-10 producing CD4+ T cells. These novel findings suggest a complex role for PD-1 in regulation of the chronic immune response to *L. infantum*. Recovery of a productive and efficacious CD4+ T lymphocyte response in VL would improve the efficacy of current therapeutic options and reduce the duration of treatment necessary to achieve remission of clinical disease and parasitological cure.

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CONTROL OF PARASITE GROWTH VERSUS PATHOLOGY IN *LEISHMANIA BRAZILIENSIS* INFECTION

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Cutaneous leishmaniasis (CL) and mucosal leishamaniasis (ML) are characterized by high production of pro-inflammatory cytokines and development of pathology. In contrast, 70% of the individuals exposed to *Leishmania braziliensis* infection do not develop disease. Sub-clinical *L. braziliensis* infection (SC) is characterized by a positive delayed type hypersensitivity test (DTH) and production of lower amount of IFN-γ and TNF-α than CL and ML patients. Here we evaluate why individuals with SC *L. braziliensis* infection had a weak type 1 immune response and how they control leishmania infection. Cytokines (IL-10 and IL-23) and neutralizing antibodies anti cytokine were added to lymphocyte cultures. The ability of macrophages from individuals with different clinical forms of *L. braziliensis* infection to produce chemokines and kill *L. braziliensis* was determined. Cytokines and chemokines were measured in lymphocyte and macrophage cultures by ELISA and PCR. The production of IL-10 and IL-27 were not enhanced in SC and neutralization of IL-10 did not enhance IFN-γ production in these individuals. While macrophages from CL and ML patients produced higher amounts of CXCL9, CXCL10 and TNF-α than SC subjects, killing of *L. braziliensis* was higher in SC individuals than in CL and ML patients. These data show that macrophages and lymphocytes from CL and ML patients produce higher levels of pro-inflammatory chemokines and cytokines that were associated with pathology. In contrast, macrophages from SC individuals kill more efficiently *L. braziliensis* than macrophages from CL and ML, indicating that protection is associated with the innate immune response.

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GROWTH KINETICS AND CELL VIABILITY OF FIVE REFERENCE STRAINS OF *LEISHMANIA* FROM THE WORLD HEALTH ORGANIZATION

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Leishmaniasis is a public health problem in countries with endemic zones. Since 1972 the reference strains of the WHO have been used in *Leishmania* research. Once these strains are acquired, they are cultivated and amplified without knowing relevant information, like growth kinetics, which could affect the diagnosis of the disease and the associated research. The aim of this study was to characterize the growth kinetics and cell viability of five reference strains of *Leishmania* obtained from the WHO and to determine changes in growth kinetics between the evaluated

species and along passages. The growth kinetics was performed in the passages 1, 5 and 10 by determining promastigote daily concentration and viability, using propidium iodide staining and the software CellProfiler until a zero viability was obtained. The evaluated species were: *Leishmania braziliensis* (MHOM/BR/75/M2903), *L. panamensis* (MHOM/PA/71/LS94), *L. guyanensis* (MHOM/GF/79/LEM85), *L. amazonensis* (MHOM/BR/73/M2269) y *L. mexicana* (MHOM/BZ/82 BEL 21). The results showed that the species of the subgenus *Leishmania* reached the maximum growth between the third and fourth day of culture, while species of the subgenus *Viannia* did it at sixth and seventh day of culture. Differences between passages 5 and 10 were not found among almost all growth kinetic curves. However, after comparing these two similar passages with passage 1 it was found that the final concentration of parasites at the end of their logarithmic phase was twofold higher in passage 1 for *L. amazonensis* and a half fold for *L. guyanensis* and *L. mexicana*. For the other species there were not differences between passages. Once the logarithmic phase had come to its end, the parasites viability decreased from 100% to values near to 0%. In conclusion, we found differences in the growth kinetics and viability of the parasites between the subgenus *Leishmania* and *Viannia* but not among the species of each subgenus. We found differences in the growth kinetics between the passages, but patterns were different for each species.

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CPG (TLR9) MEDIATED IMMUNOTHERAPY OF CHRONIC *LEISHMANIA (VIANNIA) PANAMENSIS* INFECTION IN THE MOUSE MODEL

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Leishmania (Viannia) are the primary agents of cutaneous leishmaniasis in the Americas. New therapeutic approaches are necessary, as current treatment strategies are hindered by severe side effects, lengthy treatment regimens and emerging drug resistance. Patients infected with L. (V.) panamensis display a mixed Th1/Th2 cytokine profile, which is replicated in a chronic infection BALB/c mouse model. Utilizing this model, the therapeutic potential of the TLR9 agonist, unmethylated CpG, which is known to promote a Th1 cytokine response, was evaluated. Mice, with established lesions, treated with CpG, had significantly reduced lesions compared to controls. Surprisingly, when the draining lymph node response was analyzed directly after treatment, there was significantly reduced IFNγ, IL-10, IL-13 and IL-17 in treated mice. Further, an increased IL-10:IFNγ ratio was observed. Consistent with these observations, *in vitro* experiments revealed that draining lymph node cells from infected mice, when treated with high (1uM) doses of CpG in the presence of soluble leishmanial antigen, produced reduced IFNγ and increased IL-10 responses. These results suggested that down-regulation of immune and inflammatory responses were involved in disease amelioration. To test this, infected mice were treated with the indoleamine 2,3-dioxygenase (IDO) inhibitor, 1-methyl tryptophan (1-MT), which is known to inhibit regulatory T cell development. Mice treated with 1-MT, developed larger lesions, higher parasite burdens, and increased cytokine production of IFNγ, IL-10, IL-13 and IL-17. These results are consistent with studies utilizing CpG treatment in autoimmune disease and suggest that CpG may act by inducing a beneficial Treg response that dampens lesion site cellular recruitment and pathology of leishmaniasis. Further studies are in progress to further evaluate the CpG regulatory response to determine if this may provide an alternative approach to current chemotherapeutic treatment.

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FREQUENCY OF LYSOSOME DEPENDENT AND INDEPENDENT CELL ENTRY BY THE TCI LINEAGE OF *TRYPANOSOMA CRUZI*

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Chagas disease is caused by *Trypanosoma cruzi*, an obligate intracellular protozoan parasite. An essential part of *T. cruzi's* life cycle is the process of cell invasion, which is required for parasite multiplication inside its mammalian host. *T. cruzi* is capable of invading non-phagocytic host cells through two different mechanisms. In the "lysosome-dependent pathway" (LDP), the parasite enters the host cell surrounded by vacuoles derived from host cell lysosomes. In contrast, in the "lysosome-independent pathway" (LIP), *T. cruzi* enters the host cell enveloped in a plasma membrane-derived vacuole, which only later acquires lysosomal markers. Recent studies based on *in vitro* assays show that the LIP is more frequently used by Y-strain (TcII lineage) parasites. However, *T. cruzi* is a highly heterogeneous species, whose six distinct lineages (TcI-TcVI) differ widely in their biological properties. At present, information about frequency of use of each cell invasion mechanism by other *T. cruzi* lineages is not available. We measured the infectivity and the frequency of usage of each invasion mechanism by *T. cruzi* parasites belonging to the TcI lineage (three different isolates and the Brazilian strain). Although we found large differences among isolates regarding *in vivo* and *in vitro* infectivity, we determined that the frequency of usage of each of the two invasion routes is very similar to those of the TcII lineage (Y-strain). Our results suggest that the frequency with which each cell invasion mechanisms is used by different isolates is independent of their degree of infectivity. In addition, they suggest that preference for the LIP invasion route is independent from the parasite lineage.

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*LMEX*NUC-1 AND *LMEX*NUC-2: TWO FUNCTIONALLY IMPORTANT SECRETORY/RELEASED NUCLEASES OF *LEISHMANIA MEXICANA*

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Leishmaniasis affects 2 million people worldwide; its symptoms range from localized cutaneous lesions to systemic disease. *Leishmania* promastigotes (Pro) are transmitted to mammals via the bite of an infected sand fly. In the mammalian host Pro transform into amastigotes (Am) which reside and multiply within macrophage phago-lysosomal vacuoles. All *Leishmania* species are purine auxotrophs; i.e. they need to acquire these essential molecules from their mammalian/insect hosts. Thus it is relevant to investigate the purine scavenging pathways and enzymes of this unicellular parasite. Using molecular biology techniques we demonstrated that *L. mexicana* release/secrete two 35kDa nucleases-nucleotidases: *Lmex*NUC^{s}-1 and -2. The two enzymes are located on chromosome 29 and are 95% identical. RT-PCR and Northern-blot analyses showed that expression of *Lmex*NUC^{s}-1 and -2 is higher in Am compared to Pro. Over-expression experiments and confocal microscopy showed that both, *Lmex*NUC^{s}-1 and -2 are synthesized by amastigotes while inside macrophage phago-lysosomal vacuoles. The tertiary protein structure of *Lmex*NUC^{s}-1 and -2 chimeras including several disulfide bonds and a metal co-factor (Zn^{2+}) were found essential for enzyme function. Anti-sense experiments suggested that *Lmex*NUC^{s}-1 and -2 were not absolutely essential for parasite survival *in vitro* using single polynucleotide purine substrates. However, such anti-sense transfectants showed that *Lmex*NUC^{s}-1 and -2 are necessary for parasite invasion/survival in J774 mouse macrophages. Our cumulative results suggest that *Lmex*NUC^{s}-1

and -2 play important role(s) in facilitating the growth, development and survival of this important human pathogen. Thus, these enzymes may represent logical targets for therapeutic intervention,

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FUNCTIONAL CHARACTERIZATION OF A GENE ENCODING A UNIQUE DEVELOPMENTALLY EXPRESSED SECRETORY INVERTASE FROM *LEISHMANIA MEXICANA*

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Leishmania parasites are all transmitted by phlebotomine sand flies. Within these vectors, these parasites multiply and move anteriorly in the sandfly alimentary tract. During this migration these parasites must obtain host derived nutrients/energy sources to survive and multiply. Sand flies characteristically ingest plant sugars, including sucrose and other polysaccharides, and store these sugars in their crop. Between blood meal feeds they regurgitate such sugars into their anterior mid-gut. In that regard, recently, we found that *L. mexicana* promastigotes (Pro) secrete/release an invertase/sucrase activity into the culture medium during growth *in vitro*. In contrast, *L. mexicana* axenic amastigotes (AxAm) do not release any detectable invertase activity. To characterize this invertase activity further, we adopted a molecular approach. Using PCR methods, we identified a gene which encodes a putative *L. mex* invertase (LmxM04.0310; *LmxINV*). Results of RT-PCR demonstrated that mRNA for LmxINV was expressed only by *L. mex* Pro and was not detected in *L. mex* AxAm. The *LmxINV* encodes a 71.5 kDa protein with conserved β-fructofuranosidase domains and a secretion signal peptide. To characterize this enzyme further, we designed an expression constructs containing a C-terminal hemagglutinin tag (*LmxINV:HA*). This was ligated into the leishmanial episomal expression vector pKSNEO. Following electroportation, *L. mex* transfectants were selected for growth in increasing concentrations of G418. Results of enzyme assays demonstrated that such transfectants expressed more than 100 fold higher levels of secreted invertase activity than vector-match controls. Such activity was readily immuno-precipitated with anti-HA monoclonal antibody beads. Western blots of such immuno-precipitates showed only a single ~72 kDa band of LmxINV:HA protein. Such transfectants were readily propagated as promastigotes but were incapable of true transformation into axenic amastigotes *in vitro*. These results suggest that LmxINV has additional roles in developmental biology of this human pathogen.

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HISTOPATOLOGICAL CHANGES IN CARDIAC TISSUE FROM *CAVIA PORCELLUS* EXPERIMENTALLY INFECTED WITH *TRYPANOSOMA CRUZI*

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We have previously shown that guinea pigs infected with *Trypanosoma cruzi* develop histopathological changes with similar characteristics to the human Chagas disease. Our aim was determine whether variations in levels of collagen I, III and IV are related to the parasite load and the degree of inflamation in the cardiac tissue, during the experimental infection of guinea pigs by *T. cruzi*. Seventy-two guinea pigs were inoculated intradermally with 10^4 trypomastigotes of *T. cruzi* strain Y (experimental group, EG), and 18 guinea pigs were used as a control group (CG). Eight animals from EG and two from CG were sacrificed at 5, 15, 20, 25, 40, 55, 115, 165 and 365 days post infection. The immunotypes of collagen (CI, CIII and CIV) were detected by immunohistochemistry. We observed a decrease in levels of collagen I during the acute phase (20-55 days pi) and it was associated with high numbers of amastigote nests and severe inflammation. During the early chronic phase (115-165 days pi) there was a slight increase in the levels of the three types of collagen examined. An increase in levels of CI, CIII and CIV was observed during the chronic phase (365 days pi), where CIII and CIV had the highest levels. Also in this phase, CI and CIII were detected in interstitial and perivascular spaces and CIV was detected in some interstitial forms and on the basement membrane of cardiomyocytes. The deposits of collagen were associated with inflammatory cells such as lymphocytes and some macrophages, suggesting an association of the inflammatory process with fibrogenesis in chronic Chagas disease. Additionally, during that phase, the fibrosis in cardiac tissue was associated with the presence of parasite DNA. These results show the usefulness of the guinea pig as an animal model to explain cardiac remodeling during *T. cruzi* infection.

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PATHOGENICITY OF *PLASMODIUM FALCIPARUM* FIELD ISOLATES AND INHIBITION OF HUMAN ENDOTHELIAL CELL APOPTOSIS BY FASUDIL

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Plasmodium falciparum infection can abruptly progress to severe malaria, a life-threatening complication resulting from sequestration of parasitized red blood cells (PRBC) in the microvasculature of various organs such as the brain and lungs. PRBC adhesion can induce endothelial cell (EC) activation and apoptosis, thereby disrupting the blood-brain barrier. Moreover, the hemozoin, the malarial pigment induces the erythroid precursor apoptosis. Despite the current efficiency of antimalarial drugs in killing parasite, severe malaria still causes up to one million deaths every year. A new strategy targeting both parasite elimination and EC protection is urgently needed in the field. Recently, a rho-kinase inhibitior Fasudil, a drug already in clinical use in human for cardio- and neuro-vascular diseases, was successfully tested on laboratory strains of *P. falciparum* to protect and to reverse damages of the endothelium. We therefore assessed herein whether Fasudil, would have a similar efficiency on *P. falciparum* taken directly from malaria patients using contact and non contact experiments. Seven (23.3%) of 30 PRBC preparations from different patients were apoptogenic, four (13.3%) acting by cytoadherence and three (10%) via soluble factors. None of the apoptogenic PRBC preparations used both mechanisms indicating a possible mutually exclusion of signal transduction ligand. Three PRBC preparations (42.9%) induced EC apoptosis by cytoadherence after 4 h of coculture ("rapid transducers"), and four (57.1%) after a minimum of 24 h ("slow transducers"). The intensity of apoptosis increased with time. Interestingly, Fasudil inhibited EC apoptosis mediated both by cell-cell contact and by soluble factors but did not affect PRBC cytoadherence. Fasudil was found able to prevent endothelium apoptosis from all the *P. falciparum* isolates tested. Our data provide evidence of a strong anti-apoptogenic effect of Fasudil and show that endothelial cell-*P. falciparum* interactions are more complicated than previously thought. These findings may warrant clinical trials of Fasudil in severe malaria management.

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CALCIUM AND ERYTHROCYTE INVASION DURING SICKLE CELL ANEMIA

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Regulation of intracellular Ca^{2+} is essential for *Plasmodium falciparum* development and survival and invasion of the red blood cell (RBCs) can induce senescence. In the same line increase of the Ca^{2+}level in RBCs can trigger eryptosis. In RBCs with sickle cell trait (HbSS) Ca^{2+} concentration is thus considerably higher than in normal ones (HbAA) and is associated with premature senescence and eryptosis. This can limit the lifespan of the parasite in patients with sickle cell anaemia and may explain their relative protection against malaria. However recent studies warn that children with sickle cell anaemia are more likely to die from severe malaria, suggesting that parasites can survive within this hostile environment. To deal with this topic, we use homozygote HbSS RBCs to study invasion and development of *P. falciparum* during sickle cell anaemia. Using FLUO4-AM we compare the calcium content of HbSS and HbAA infected RBC. Over 72h of culture, we found that parasite grow slower in HbSS RBCs than in HbAA ones with a delay in the life cycle. Using Q-PCR we found no change in PfATP6, PfATP4, PfV1, PfV2, PfCAX, PfNHE expression between the two set of parasites. By flow cytometry we found a great heterogeneity of Ca^{2+} level in HbSS RBCs, with 21.2% harbouring a high concentration of Ca^{2+}. Double staining of parasitized erythrocytes with Fluo4-AM and hydroethidine showed no significant difference in the Ca^{2+} content of HbAA and HbSS parasitized RBCs (Fluo4 mean of fluorescence 3.33 and 4.40 respectively). These results draw question about whether parasites infect only HbSS RBC with a normal intracellular Ca^{2+} level or whether they regulate the Ca^{2+} content of the RBC early after invasion without evidence of modulation of the main cation exchanger expression.

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AN ENDOGENOUS NITRIC OXIDE SYNTHASE INHIBITOR IS ASSOCIATED WITH SEVERE MALARIA IN GAMBIAN CHILDREN

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Low nitric oxide (NO) bioavailability contributes to systemic endothelial dysfunction in severe malaria. NO generation by nitric oxide synthase (NOS) requires arginine as a substrate and is inhibited by asymmetric dimethylarginine (ADMA). The ratio of arginine to ADMA determines NO production by NOS. We hypothesized that the ratio of arginine to ADMA in blood plasma would be decreased in Gambian children with severe malaria. We enrolled children with malaria at community health centers near Fajara, The Gambia. We classified patients as mild, moderately severe (prostration), or severe (coma, respiratory distress or severe anemia) using clinical criteria. Arginine and ADMA were measured at admission and 28 days after discharge. We studied 102 mild, 45 moderately severe, and 51 severe malaria patients. The plasma arginine concentration was decreased among patients with either moderately severe or severe malaria compared to patients with mild malaria (Mild: 45.0 [IQR 35.4 - 55.7], Moderate: 25.5 [IQR 20.4 - 39.2], Severe: 32.8 [IQR 25.6 - 40.7] µmol/L, $p \leq 0.001$). The plasma ADMA concentration was elevated specifically in patients with severe malaria (0.44 [IQR 0.37 - 0.60] µmol/L) compared to patients with mild malaria (0.40 [IQR 0.33 - 0.47] µmol/L, $p = 0.02$) or moderately severe malaria (0.34 [IQR 0.27 - 0.43] µmol/L, $p = 0.002$). Plasma arginine was positively correlated with plasma ADMA; therefore, we analyzed the ratio of Arginine to ADMA. Arg:ADMA was 113.5 (IQR 86.3 - 138.2) in children with mild malaria, 82.9 (IQR 68.2 - 93.7) in children with moderately severe malaria, and 62.9 (IQR 52.9 - 79.3) in children with severe malaria ($p \leq 0.001$ for each comparison). 28 days after discharge, the Arg:ADMA ratio improved substantially within each group to 174.6 (IQR 148.0 - 209.5, $p < 0.0001$) among children with mild malaria, 144.0 (IQR 100.4 - 176.7, $p < 0.0001$) among children with moderately severe malaria, and 125.1 (IQR 99.6 - 153.3, $p < 0.0001$) among children with severe malaria. Significant differences in the Arg:ADMA ratio persisted between children recovered from mild malaria and children recovered from either moderate or severe malaria ($p < 0.005$). Severe malaria in Gambian children is associated with an acute decrease in the ratio of Arginine to ADMA, a change that could diminish endothelial cell nitric oxide synthesis. Therapeutics that inhibit ADMA release or increase ADMA clearance may help to restore endothelial function in children with severe malaria.

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TOWARDS UNDERSTANDING THE PATHOPHYSIOLOGY OF RETINOPATHY NEGATIVE CEREBRAL MALARIA BY COMPARING RATES AND TYPES OF VIRAL COINFECTIONS IN MALAWIAN CHILDREN WITH OR WITHOUT MALARIA PARASITEMIA

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The underlying pathophysiology of retinopathy negative cerebral malaria (CM) is unknown. Determining the rates and types of viral (co)infectors in patient with and without malarial parasitemia may help eludicte the role of viral (co)infection in the pathophysiology of this condition. We analyzed blood and CSF samples in three groups to compare rates and types of viral (co)infectors: retinopathy negative CM; retinopathy positive CM; and children in coma without circulating malaria parasites. The rates of viral (co)infection differed among the three studied groups. These findings lend support to the hypothesis that viral coinfection may be important in the pathophysiology of retinopathy negative CM. Our laboratory analyses had several limitations and future studies may better characterize rates and types of viral (co)infections in these three groups. Expansion of the numbers of patients sampled may further improve understanding of the pathophysiology of retinopathy negative CM.

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MALARIA PATHOGENESIS: MICROFLUIDIC MODELING OF SPLENIC FILTRATION IN MALARIA PATIENTS AT A CLINICAL FIELD SITE

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Splenic filtration is hypothesized to contribute to the pathogenesis of complicated malaria infections. We developed a method for estimating splenic filtration during malaria infection based on characterizing red blood cell (RBC) populations in peripheral circulation. Using microfluidic devices, we measured the surface area and volume of thousands of individual red blood cells from each patient. The minimum cylindrical diameter can be calculated from the surface area and volume. This parameter describes the smallest tube or pore that a cell can traverse without lysing. The minimum cylindrical diameter is useful in describing the probability of a cell becoming filtered by the spleen. This idea centers on the concept that cell geometry and not the dynamics of deformation are important to filtration of RBCs in the spleen. If the spleen is constantly filtering RBCs by their minimum cylindrical diameter, then the dimensions

of the circulating RBC population can describe splenic filtration. By measuring thousands of parasitized and normal RBCs in an individual, we can derive a model to estimate splenic filtration of parasitized RBCs in that individual. We applied this modeling technique at a field site in Blantyre, Malawi to determine if there are innate differences in an individual's RBCs or splenic filtration that could affect the presentation of malaria infection. We quantified differences between parasitized RBCs observed in peripheral circulation to those grown in culture. We observed samples from 120 individuals classified into 4 groups: cerebral malaria, uncomplicated malaria, aparasitemic coma, and healthy controls. We were able to see statistically significant differences in the uninfected RBCs from healthy controls and malaria patients. We did not see differences in the estimated splenic filtration rates between cerebral malaria and uncomplicated malaria patients. This is the first field-study where statistically significant sizes of patient populations have been analyzed with microfluidic devices to understand physiological variations between living individuals.

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AN N-ETHYL-N-NITROSOUREA (ENU)-INDUCED MUTATION IN JAK3 PROTECTS AGAINST CEREBRAL MALARIA BUT CAUSES SUSCEPTIBILITY TO MYCOBACTERIA

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Cerebral malaria (CM) is an acute, often lethal neurological complication of malaria. The cell and molecular pathways involved in CM pathogenesis are poorly characterized and need to be better understood to identify novel therapeutic targets for intervention. CM can be modeled in mice by infection with *Plasmodium berghei* ANKA. To identify genes and proteins involved in CM pathogenesis and whose inhibition may be of clinical value, we set up a forward genetic screen in ENU-mutagenized F2 mice to identify recessive mutations that protect mice against *P. berghei*-induced CM. We identified a pedigree (P48) segregating a resistance trait (in 31% of progeny) whose protective effect was fully penetrant on C57BL/6J and 129S1 genetic backgrounds, and that was mapped to the central portion of chromosome 8. Whole genome sequencing of CM-resistant P48 animals identified homozygosity for a missense mutation (W81R) in the Band 4.1/Ezrin/Radixin/Moesin (FERM) domain of the Janus-associated kinase 3 (Jak3) protein. The causative effect of W81R was verified by complementation testing, with $Jak3^{-/W81R}$ double heterozygotes being fully protected against *P. berghei*-induced CM. Immunological characterization of $Jak3^{W81R}$ homozygotes showed defects in thymic development, with concomitant severe depletion of $CD8^+$ T cells, B cells and NK cells. There was also defective T cell-dependent production of IFN- upon mitogen stimulation. Adoptive transfer of infected splenocytes from *P. berghei* infected C57BL/10J mice abrogated CM resistance in $Jak3^{W81R}$ homozygotes, an effect largely attributed to the $CD8^+$ T cell compartment. Paradoxically, $Jak3^{W81R}$ homozygotes were highly susceptible to mycobacterial infections with *Mycobacterium bovis* (BCG), *M. tuberculosis* and *Citrobacter rodentium* whose resolution depends on a robust Th1 immune response. These findings highlight the pathological role of $CD8^+$ T cell and IFN-γ-dependent Th1 responses in CM pathogenesis. They identify a direct role for Jak3 in this process, and suggest possible novel strategies for intervention in CM.

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SURVIVAL OUTCOME AND IMMUNOLOGICAL MECHANISMS OF CO-INFECTION OF SCHISTOSOMIASIS AND MALARIA IN A PRIMATE MODEL

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Multiple infections are a common phenomenon in developing countries especially Africa. Human studies have established that this is particularly true for malaria and schistosomiasis, since the epidemiological distribution of both diseases overlap. In order to determine the effect of multiple infections on disease outcome, we conducted a controlled experiment to investigate the effect of chronic schistosomiasis on severe malaria (SM) and acquired immunity to malaria infection in the baboon. To understand the underlying mechanisms, we analyzed immunological parameters associated with SM during co-infection and determined how worm infection affects development of acquired immunity to malaria. The experiment was conducted in two phases; effect of chronic schistosomiasis on severe malaria (phase 1) and acquired immunity (phase 2). In phase 1, four groups of baboons were used. Groups A, B and C were infected with 500 *Schistosoma mansoni* cercarie. To determine the effect of treatment on co-infection, group A was treated with praziquantel at week 14 and 15. Four weeks later, groups A, B and D were inoculated with 105 *Plasmodium knowlesi* parasites. In phase 2, three groups of baboons were used. Groups A and B were subjected to the same procedure as B and C above. However after schistosome treatment all groups were infected twice with *P. knowlesi*, cured with artemether/lumefantrine at 2% parasitaemia and challenged a third time. Results from phase 1 showed that *P. knowlesi* infected animals had an early onset of parasitemia and succumbed to SM unlike majority of co-infected baboons. For animals with both infections, we noted elevated levels of Th2 cytokines (especially IL-6) and T-regulatory markers prior to malaria infection. In phase 2, all animals were protected from SM after multiple infection and treatment. This study demonstrates that chronic schistosomiasis reduces SM and this could be mediated by pro-inflammatory cytokines and suggests a role for T-regulatory pathways. On the other hand, the presence of schistosomiasis does not affect acquired immunity to malaria infection in baboons.

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THE IMMUNOMODULATORY ACTIVITY OF MALARIA PIGMENT (HEMOZOIN) ON THE INNATE IMMUNE RESPONSE: PLATELETS AND MONOCYTES AS KEY EFFECTOR CELLS

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Malaria has profound worldwide impacts, infecting millions of people annually with the highest mortality rates occurring in the children of sub-Saharan Africa infected with *Plasmodium falciparum*. The parasite's life cycle occurs within infected erythrocytes and produces hemozoin, a crystalline metabolite of hemoglobin digestion, which is released during malaria infection and found in high concentrations in the circulation. The effects of the key malarial toxin on human platelet response have not been examined. Therefore, we characterized the interaction of pure, synthetic hemozin (sHz) on human platelets and monocytes *in vitro*. We observed that surface P-selectin and PAC-1 binding were increased when human platelets were stimulated of sHz (2-20μM) and that sHz (2μM) potentiated platelets activation by thrombin (0.01 μ/mL). In addition, sHz also induced release of platelet factor 4 and RANTES by human platelets. Futhermore, sHz increased golgi apparatus compared to thrombin activation based on transmission electron microscopy and immunocytochemsitry, suggesting that sHz alters post-translational processing of proteins in platelets. We also found that sHz triggers platelet aggregates and formation of heterotypic aggregates with human monocytes, a sensitive marker of

platelet activation. Platelet-monocyte aggregates formed in whole blood and in isolated cell suspension in response to sHz. This was interrupted by a blocking antibody against P-selectin indicating that binding P-selectin to P-selectin glycoprotein ligand 1 (PSGL-1) on the monocyte which mediates both adhesion and signaling is a key mechanism. Our observations demonstrated that sHz is an agonist for platelet activation and interactions with monocytes, that it may have important roles in platelet-mediated events in clinical malarial syndromes.

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We also found that sHz triggers platelet aggregates and formation of heterotypic aggregates with human monocytes, a sensitive marker of platelet activation. Platelet-monocyte aggregates formed in whole blood and in isolated cell suspension in response to sHz. This was interrupted by a blocking antibody against P-selectin indicating that binding P-selectin to P-selectin glycoprotein ligand 1 (PSGL-1) on the monocyte which mediates both adhesion and signaling is a key mechanism. Our observations demonstrated that sHz is an agonist for platelet activation and interactions with monocytes, that it may have important roles in platelet-mediated events in clinical malarial syndromes.

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DELETION OF ADß2 INTEGRIN PROTECTS AGAINST EXPERIMENTAL CEREBRAL MALARIA

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Malaria, a disease of major importance in many areas of the world, causes a variety of pathologic syndromes including cerebral malaria, which is often fatal. Leukocyte integrins are essential for host defense but also mediate pathologic as well as physiologic responses of the innate and adaptive immune systems. Their roles in malarial syndromes have not been defined. We previously showed that targeted deletion of the αD subunit (αD-/-) of αDβ2 integrin, which is expressed on key macrophage subsets in mice and humans, leads to absent expression of the integrin heterodimer on murine leukocytes and reduces mortality of mice infected with *Plasmodium berghi* Anka (PbA), a cause of experimental cerebral malaria. To further indentify mechanisms that are involved, we examined wild type (WT) and αD-/- mice at 7 and 10 days after PbA infection and found significant decreases in vessel plugging, micro-hemorrhages, and necrosis in the brains of αD-/- animals. Intravital microscopy and flow cytometry demonstrated lower numbers of rolling and adherent leukocytes in cerebral vessels of αD-/- mice, and decreased T lymphocyte accumulation in the brains of infected animals. Evans blue dye exclusion assays demonstrated significantly less dye extravasation in the brains of αD-/- mice at day 10, indicating preserved blood-brain barrier integrity. Furthermore, there were altered patterns of inflammatory cytokine expression in αD-/- compared to WT mice at 7 and 10 days. Neurophysiologic analysis indicated that αD-/- mice had improved cognitive function, which may result from reductions in brain vasculopathy, leukocyte sequestration, and inflammation. We conclude that deletion of αDβ2 alters the natural history of severe experimental malaria, demonstrating previously-unrecognized activities of a key leukocyte integrin in immune and inflammatory responses in this syndrome.

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CHARACTERIZATION OF A NOVEL FAMILY OF LONG NON-CODING RNA TELOMERE-ASSOCIATED REPETITIVE ELEMENT (LNCRNA-TARE) TRANSCRIPTS IN *PLASMODIUM FALCIPARUM*

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The importance of long non-coding RNAs (lncRNAs) in epigenetic remodeling and transcriptional regulation has been recently established across numerous eukaryotic systems. Given *Plasmodium falciparum*'s susceptibility to histone deacetylase inhibitors and mounting evidence for epigenetic regulation of multi-gene virulence families, we hypothesized that lncRNAs are involved in the *P. falciparum* transcriptional network. Using a high-resolution DNA tiling microarray, we have identified and characterized several putative *P. falciparum* lncRNAs, including a novel family of twenty-two long non-coding RNA telomere-associated repetitive element (lncRNA-TARE) transcripts. LncRNA-TARE transcripts are coordinately expressed after parasite DNA replication from at least eighteen chromosome termini and encompass the majority of known binding sites (SPE2) for the ApiAP2 transcription factor PfSip2. Interestingly, the SPE2 binding site is only otherwise found in the promoter of *upsB*-type *var* genes, and an *upsB*-type *var* gene is adjacent to each lncRNA-TARE gene. LncRNA-TARE is thus poised to play an important role at *P. falciparum* chromosome ends.

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ELEVATED SERUM HEME OXYGENASE-1 LEVELS ARE ASSOCIATED WITH NEUROLOGIC PROTECTION IN CHILDREN WITH CEREBRAL MALARIA

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Heme oxygenase-1 (HO-1) degrades heme into biliverdin, carbon monoxide and free ferrous iron and acts as a regulator of pro- and anti-inflammatory cytokine activity. HO-1 is associated with protection from development of experimental cerebral malaria in murine models. HO-1 protection to malaria is thought to be due to effects against inflammation and oxidative stress. To further investigate the role of systemic HO-1 in cerebral malaria (CM) pathogenesis, we have measured serum HO-1 levels in Ugandan children with CM (n=74), uncomplicated malaria (UM, n=68), and healthy community children (CC, n=60). HO-1 levels (median [interquartile range], ng/mL) were higher in Ugandan children with CM (28.4 [61.4]) than in children with either UM (16.5 [54.3], *P*=0.05) or

community children (8.3 [8.3], *P*<0.0001). Children with CM who did not have neurologic deficits at discharge or at 3-month follow-up had higher admission serum HO-1 levels than those who did have deficits at those time points (discharge: 36.1 [83.0] vs 23.4 [14.5], *P*=0.07; 3-month follow-up: 28.7 [61.6] vs 14.4 [2.2], *P*=0.03). Elevated serum HO-1 levels were not associated with protection from death. HO-1 levels are elevated in children with CM and are associated with neuroprotection in these children.

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UMBILICAL AND UTERINE ARTERY DOPPLER STUDIES AMONG MALARIA INFECTED AND NOT INFECTED PREGNANT WOMEN

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Malaria complicated pregnancies are a significant public health problem affecting mothers and their offspring. In this longitudinal cohort study, our goal was to determine if abnormalities *in utero*placental blood flow were indicative of a malaria complicated pregnancy. Pregnant women were recruited from Msambweni Kenya, at the time of their first antenatal visit. Patients with known medical disorders contributing to fetal growth restriction, placental dysfunction, and prematurity were excluded. Using a SonoSite 180 Plus ultrasound machine, the uterine and umbilical artery Doppler indices were studied in addition to fetal biometrics. Malaria infection was determined by PCR from maternal blood samples taken at the time of the first clinic visit and at delivery (maternal venous, placental-intervillous, and cord blood). Newborn birth weight, length, and head circumference were measured. Study outcomes were stratified by 3 week gestational age groups and compared in malaria infected vs. not infected women. 471 women were enrolled. Malaria prevalence for study participants was ~7%. In 18-23 week gestational age groups, women with malaria infections had increased umbilical artery pulse index (PI), resistance index (RI) and systolic/diastolic (S/D) ratios compared to women not infected with malaria. This effect was not seen in later gestational age groups. No difference in uterine artery Doppler indices was found between malaria infected and not infected women. These umbilical artery abnormalities were not observed in later periods of pregnancy. Doppler measured abnormalities may be indicative of malaria complicated pregnancies. Given the small sample size, further research is needed. Malaria prophylaxis should be encouraged in all pregnant women as early as possible in pregnancy.

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ORIGIN OF A LINAGE OF *PLASMODIUM* SPECIES IN ORANGUTAN

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The large number of *Plasmodium* samples recently obtained from African Apes has provided new perspectives on the evolution of human and ape malarias. A missing piece of the puzzle, however, are the malarias found in Apes from Southeast Asia. In this study, we report molecular data for a malaria parasite lineage found in orangutans. Twenty four blood samples were collected in 2003 at a "Orangutan Care Center and Quarantine (OCC&Q)" in Indonesia. We screened the samples for *Plasmodium* parasites by nested PCR using the cytochrome b (cyt b) gene. For all positive samples, parasite mitochondrial genomes (mtDNA) and two antigens: circumsporozoite protein gene (CSP) and merozoite surface protein 1 42kDa (MSP-142), were amplified, cloned, and sequenced. We found 15 orangutans positive by PCR using cytb primers. These isolates yielded five distinctive mitochondrial haplotypes not previously found in non-human primates and were found to exhibit low genetic divergence among them suggesting that they belong to one species. Whereas positive blood smears were available, we could not establish whether they belong to any of the two previously described orangutan malarias. We report a phylogenetic analysis that includes this parasite from orangutan using complete mitochondrial genomes, CSP and MSP-142 separately. Our phylogenetic analyzes revealed that the orangutan malaria lineage was part of a monophyletic group that includes all the known non-human primate malarias found in Southeast Asia; specifically, it shares a recent common ancestor with *P. inui* (a macaque parasite) and *P. hylobati* (a gibbon parasite). This finding suggests that this lineage may have originated as a result of a host switch from a non-Ape host. As has been previously observed in the other *Plasmodium* species found in non-human primates, the CSP protein shows high polymorphism in the number of repeats. The polymorphism found in the non-repetitive 3' and 5' regions is similar to the one reported for other parasites and appears to be neutral. In contrast, the genetic diversity of MSP-142 in orangutan is twice that observed in *P. vivax* and seems to be under positive selection. This result is similar to previous findings in non-human primate malarias closely related to *P. vivax*. The limited molecular evidence available from Asian Apes indicate that these parasites originated independently from those found in Africa, likely as the result of host switches from other non-human primates.

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IN THE PERUVIAN AMAZON, MALARIA INFECTION SEVERITY IS ASSOCIATED WITH HOST AND PARASITE VARIABLES AND GROWTH *IN VITRO*

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It is widely reported that not all *Plasmodium falciparum* isolates are capable of adapting to culture. We looked for variables that predict *in vitro* growth considering 1663 *P. falciparum* infections of which 274 were put directly into culture. Parasite growth was measured by *ex-vivo* parasite multiplication rate (ex-PMR, fold increase in parasitemia between generations) and overall culture success over 21 days. Variability in parasite genotype was investigated for associations with disease parameters and *in-vitro* growth using 14 microsatellite markers and polymorphisms in the Merozoite Surface Protien-1 block 2 (MSP1-B2). We considered *in vitro* growth dynamics using a novel quantitative PCR method, which was validated by comparing 80 clinical with nested PCR and capillary gel electrophoresis techniques. Parasite density was associated with presence or absence of fever, complexity of infection, microsatellite cluster, ex-vivo PMR and growth success ($p<0.0001$ for all associations). There was an inverse correlation between starting in-vitro parasitemia and ex-PMR during the first 48 hours (low parasitemia PMR=1.7, high parasitemia PMR= 2.5; $p=0.0002$). We suspect this ex-PMR difference is related to host immunity by 1) residual antibodies from high density infections inhibiting *in vitro* growth or 2) a fast growing parasites infecting immune individuals but the growth only being observable when removed and placed into culture. Independent of density, we found evidence for separate independent associations between febrile illness and complexity of infection with isolate stability *in vitro*. Using qPCR we found that complex infections provided a density stabilizing dynamic in the cultures: single clone infections could growgrew faster but were more likely to die off. Although there was no directly predictability of ex-PMR or culture success using parasite genotype, our results suggest that host immunity

is limiting the growth parasites *in vivo* and that complex infections are more successful in culture despite there being some competition *between genotypes in vitro*.

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THE NANO-TERRAIN OF *PLASMODIUM FALCIPARUM* AND *P. MALARIAE* INFECTED RED BLOOD CELLS ISOLATED FROM CLINICAL SAMPLES

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Atomic Force Microscopy was used to characterize the nano-terrain of PCR-confirmed *ex vivo*-matured isolates of *Plasmodium malariae* and *P. falciparum* from Thailand. The surface of *P. malariae* infected cells are covered with dense 'spike-like' excrescences (mean height: 8 nm; mean diameter: 50 nm), which are morphologically distinct from the larger, more rounded 'knob' structures found on a *P. falciparum* infected red blood cell (mean height: 20 nm; mean diameter: 90 nm). The 'knobs' on red blood cells containing mature asexual forms of *P. falciparum* assist the infected cells to bind/sequester to the vascular endothelium under shear flow conditions and thus, avoid splenic clearance. The function of the *P. malariae* spikes (which were observed on every sexual and asexual stage examined) remains unknown, but it is unlikely to be used for cytoadhesion because there is no evidence that this parasite sequesters.

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FETAL AND MATERNAL HEMODYNAMICS DURING ACUTE MALARIA: PERSISTENT MATERNAL TACHYCARDIA AFTER RECOVERY FROM MALARIA

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Studies on malaria in pregnancy often focus on the effects of chronic placental malaria on the foetus. The maternal and foetal hemodynamics during acute malaria ware never studied properly. The time course of the maternal and fetal heart rate (MHR & FHR) and maternal blood pressure (BP) were studied during acute malaria until 56 days after initiation of treatment with artemether-lumefantrine (AL). We examined 38 pregnant women with acute malaria and 39 healthy pregnant control women. Malaria patients were hospitalized until recovery with a minimum of 3 days. FHR was measured every 4 hours on the first day and every 8 h for another two days and thereafter weekly. Maternal vitals were measured every 8 h for 3 days. Control women were examined once a week on out patient basis.Mean baseline characteristics of malaria patients compared to healthy women were respectively: gestational age (wks) 28.8 and 24.6 (p-value 0.006); maximum FHR (bpm) 165 and 158 (p-value 0.054); minimum FHR (bpm) 137.6 and 128.7 (p-value 0.016); mean BP (mm Hg) 75 and 81 (p-value 0.001); pulse pressure (mm Hg) 40 and 42 (p-value 0.3); MHR 107 and 81 (p-value < 0.001); Geometric mean parasite count (/µl) 13795. Complete time series were collected from 29 malaria patients and 29 controls. Maternal body temperature normalized within 24 hours; BP was normal after 72 h. Surprisingly, whereas MHR in control women showed a physiological increase during the evolution of pregnancy of approximately 7 bpm between day 0 and day 56, the initially increased MHR of malaria patients declined to 94 bpm on day 7 and stabilized at this level. There were no pathological CTG records. The mean FHR normalized after 72 h. In conclusion, acute malaria induces maternal and fetal hemodynamic changes that normalize at a different pace after initiation of treatment with AL. Fetal heart rate and BP normalized between days 3 and 7 after initiation of treatment. Surprisingly, maternal heart rate remained elevated. This is yet unexplained.

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A RANDOMIZED STUDY TO COMPARE A FIXED DOSE COMBINATION OF ARTESUNATE PLUS AMODIAQUINE VERSUS A FIXED DOSE COMBINATION OF ARTEMETHER PLUS LUMEFANTRINE IN TREATMENT OF REPEATED UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA ATTACKS OCCURRING DURING TWO YEARS IN CHILDREN IN UGANDA

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Although in high-endemic areas artemisinin combination therapy (ACT) is used repeatedly by patients, very few studies document the safety of multiple ACT administrations. We designed a study to assess the safety and efficacy of repeated administrations of the fixed-dose combination artesunate + amodiaquine (ASAQ) in comparison with artemether-lumefantrine (AL) in consecutive episodes of uncomplicated *P. falciparum* malaria in children. This randomized, investigator-blinded, comparative study was conducted in a rural community of Eastern Uganda from June 2008 to June 2010. Patients under 5 years of age with uncomplicated *P. falciparum* malaria were randomized to receive either ASAQ once daily, or AL twice daily for three days for each malaria episode occurring over a period of 2 years. Treatment intake was supervised only for first episodes. All attacks were monitored until D42. A total of 413 patients were randomized in the two groups (208 ASAQ, 205 AL). During the study period, a total of 6032 malaria episodes were treated. The median number of episodes were 16 and 15 in ASAQ and AL groups respectively. Treatment-emergent AEs were reported during follow-up in 59.8 % of the patients without significant differences between the 2 groups; only one AE in each treatment group was considered as related to treatment. Adverse event of special interest (AESI) were observed in 28 patients (29 episodes); abnormalities in liver function tests were reported in 23 patients (11 ASAQ, 12 AL), and neutropenia in 6 patients (4 ASAQ, 2 AL). All AESI were reversible. Serious adverse events were reported in 25 patients (31 episodes) without any difference between the two treatment groups. Incidence of adverse events did not increase with the repetition of treatment, in either group. Efficacy analysis is ongoing. An unexpectedly high number of malaria attacks were seen in each treatment group. These results confirmed the satisfactory safety profile of ASAQ in comparison with AL, with no issue related to repeated administration.

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CHALLENGES IN ESTABLISHING A COHORT-EVENT MONITORING DRUG SAFETY STUDY IN IFAKARA AND RUFIJI HDSS, TANZANIA

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The recommended artemisinin combination therapy (ACT) for treatment of uncomplicated malaria in Tanzania is artemether-lumefantrine (AL). Although Artemisinin and its derivatives are generally thought to be safe, there is currently little or no data on its safety among populations in Tanzania. In view of this INESS established a phase IV study to evaluate safety of AL through comprehensive pharmacovigilance in large populations with the aim of documenting rare adverse drug reactions and to characterize known effects in 'real-life'. The methodology employed is cohort event monitoring which is observational, longitudinal and prospective. Patients with diagnosis of malaria for whom AL was prescribed were recruited into the cohort from four health facilities in each HDSS. Information on demographics, use of all medicines, mode of diagnosis of malaria, presenting signs and symptoms, co-diagnoses, events suspected as adverse drug reactions, reasons for stopping the drug and cause of death (if any) were collected using standardized questionnaire. They were followed up on 7 to 10 days after AL was dispensed. This report is on the number recruited so far and the challenges in getting the cohort going. 9028 patients were recruited. 9016 (99.8%) completed follow-up on day 7, of which 668 (7.4%) were done by telephone calls. 12 (0.13%) were lost during follow-up. The main challenges encountered are getting enough trained staff to recruit and follow up patients since CEM is quite labour intensive. 38 health providers and 10 field workers were recruited and offered the relevant training in collaboration with regulatory authorities. This helped to overcome the human resource challenge. Another challenge involved is the difficult to reach areas which are cut off especially during the rainy season. Follow up by telephone was adopted for these areas and this helped to reduce number of lost to follow-up. Setting up a cohort event monitoring program takes time and is demanding in terms of human resource. Training is very important in overcoming this. Involvement of all stakeholders and sponsors is a key to success.

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DRUG-DRUG INTERACTIONS BETWEEN PRIMAQUINE AND CHLOROQUINE: STUDIES USING POOLED HUMAN HEPATOCYTES

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The long established potentiation of primaquine's liver stage activity when co-administered with chloroquine is poorly understood. In the present study, we have undertaken a series of *in vitro* experiments using pooled primary human hepatocytes and recombinant isoenzymes, in order to determine whether the roots of this effect lie in the metabolism of primaquine and if there are any dose dependent inhibitory effects with chloroquine. Increasing chloroquine concentration appears to significantly inhibit primaquine metabolism. Following four hours incubation in hepatocytes an apparent dose dependent decrease in carboxyprimaquine production and production of a metabolite at m/z 261, a mass consistent with the primaquine alcohol, was observed with increasing chloroquine concentrations. This suggests that a significant inhibitory effect on the carboxyprimaquine pathway may play a role in the observed potentiation.

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METABOLITE IDENTIFICATION OF THE 8-AMINOQUINOLINE DRUG PRIMAQUINE USING RECOMBINANT HUMAN METABOLIC ISOENZYMES

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Primaquine's mechanisms of activity and toxicity have long been thought to be mediated by one or more metabolic byproducts, and the exact chemical nature of these species and their metabolic pathways is poorly understood. Previous work in our lab has determined that CYPs 2D6, 3A4, and 2C19 and Monoamine Oxidase-A (MAO-A) are major contributors to primaquine metabolism. In the present study, primaquine was incubated with recombinant versions of these enzymes, as well as CYPs 1A2 and 2C9. The samples were analyzed by LC-MS/MS for the purpose of metabolite identification. In agreement with prior literature observations, MAO-A was found to be primarily responsible for the pathway leading to the formation of carboxyprimaquine, the major observed metabolite *in vivo*. A second metabolite at m/z 261, consistent with the primaquine alcohol, was observed after incubation with MAO-A. Other metabolites, m/z 276, consistent with the hydroxylated species largely implicated in primaquine's efficacy/toxicity profile, are mediated by the CYP enzymes, predominantly 2D6.

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SAFETY OF SEASONAL INTERMITTENT PREVENTIVE TREATMENT AGAINST MALARIA WITH SULFADOXINE PYRIMETHAMINE + AMODIAQUINE WHEN DELIVERED TO CHILDREN UNDER TEN YEARS OF AGE BY DISTRICT HEALTH STAFF IN SENEGAL

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Intermittent Preventive Treatment for malaria in children (IPTc) by monthly administration of sulfadoxine-pyrimethamine plus amodiaquine (SP+AQ) is a new strategy for malaria prevention in areas of seasonal transmission. The aim of this study was to evaluate the safety and effectiveness of IPTc when delivered by district health staff on a large scale in a three rural districts in Senegal. IPTc with SP+AQ administered once per month from September to November was delivered by nine health-posts in 2008, 27 health-posts in 2009 and by 46 health- posts in 2010. Doses administered in each village were documented in a register. A surveillance system was established to record all deaths, and malaria cases were diagnosed at health facilities. Surveillance for adverse events that might be drug-related was maintained in three regional hospitals, three district health centres, 55 health posts and through active follow-up at home. Community health workers visited each child one month after the first and second rounds of treatment to check that there had been no severe reactions to the previous treatment, and to give the next round of treatment. Health staff in all facilities were sent SMS messages before each monthly treatment round to remind them to report any adverse events. All health facilities were visited monthly to check and collect adverse event reports. Admission records for all hospital inpatients admitted following IPTc administration were reviewed for evidence of a possible relationship with

drug intake. After 3 years of intervention over 980,000 documented courses of IPTc had been administered by community health workers. High coverage of three courses of treatment was achieved. No serious adverse events attributable to the intervention were reported. IPTc with SP+AQ is safe and well tolerated when implemented on a large scale. IPTc should be considered for implementation as additional malaria control intervention in areas where seasonal malaria continues to cause severe illness and mortality among children.

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STATEVILLE HUMAN STUDIES OF FORTY 8-AMINOQUINOLINES

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The 8-aminoquinoline (8-AQ) antimalarials are the only class effective against *Plasmodium vivax* relapse and *P. falciparum* gametocytes. This class is highly desirable for malaria prevention, malaria control and especially malaria elimination. However, the risk of the hemolytic toxicity has been recognized with 8-AQs. From 1945-49 at Stateville, IL, researchers evaluated, in a clinical research unit, 40 8-AQs for antimalarial efficacy and tolerability. However, in the ensuing years, much of this information was filed away and forgotten. The purpose of this effort is to compile all unpublished efficacy and safety data reports in humans. We identified, collected and extracted the historical data on efficacy and safety of the 8-AQs from unpublished reports by contacting research experts and by searching libraries where data was kept. Data were entered and analyzed in Excel (2007) and SPSS for Windows, version 16. A total of 403 clinical series (n=2,716) were identified. The majority of subjects received pentaquine (n=1,095) followed by pamaquine (n=810), isopentaquine (n=400) and primaquine (n=85). Thirty six additional 8-AQs were studied in 326 case series, including sitamaquine (WR6026). Sixteen compounds had greater than 50% efficacy reported. The most commonly reported adverse effect of 8-AQs was abdominal pain. Three subjects receiving high dose of pamaquine stopped the drug due to severe abdominal pain. Only 4 subjects had hemolytic crisis - two receiving daily pamaquine 180 mg, and two were on daily pentaquine 120 mg and 60 mg (with concurrent administration of quinine and quinacrine, respectively). Neutropenia, postural hypotension, and drug fever were identified as possible new toxicities in this class. No deaths were identified. In conclusion, this clinical safety data of 8-AQ will permit an ongoing effort to understand more clearly the structure activity and toxicity relationships of 8-AQs in humans before undertaking new synthetic efforts.

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IMPACT OF COMBINING INTERMITTENT PREVENTIVE TREATMENT WITH HOME MANAGEMENT OF MALARIA IN CHILDREN UNDER TEN YEARS, IN A RURAL AREA OF SENEGAL

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Current malaria control strategies recommend (i)Early case detection using rapid diagnostic tests (RDT) and treatment with Artemisinin Combination Therapy (ACT) (ii)Intermittent preventive treatment (iii)impregnated bed nets. However, these individual malaria control interventions provide only partial protection in most epidemiological situations. Therefore, there is a need to investigate the potential benefits of integrating several malaria interventions in reducing malaria prevalence and morbidity. We conducted a cluster randomized trial to assess the impact of combining seasonal intermittent preventive treatment in children (IPTc) with home based management of malaria (HMM) by community health workers (CHWs) in Senegal. Eight CHWs in 8 villages covered by the Bonconto health post, (South Eastern part of Senegal) were trained to diagnose malaria using RDT and provide prompt treatment with CoartemTM to children under 10 years. Four CHWs were randomised to also administer monthly IPTc with single dose of Sulfadoxine-Pyrimethamine (SP) plus three doses of Amodiaquine (AQ) in October and November 2010. A total of 1010 children in the 8 study villages were assigned to a weekly home visit by CHWs during 2 months. During each visit, an RDT was performed by CHWs for febrile children. The incidence of clinical malaria episodes was 7.1/100 child months (95%CI (3.7-13.7)) at risk in communities with IPTc+HMM compared to 35.6/100 child months (95%CI (26.7-47.4)) at risk in communities with only HMM (OR=0.20 95% CI 0.09-0.41, p=0.0001). A survey conducted at the end of the transmission season showed that malaria parasite prevalence was lower in communities with IPTc+HMM(2.05% versus 4.6% p=0.03). Adjusted for age groups, sex, *P. falciparum* carriage, prevalence of malnutrition, IPTc+HMM showed a significant protective effect against anaemia also(aOR=0.59 95% CI 0.42-0.82 p=0.02). Combining IPTc and HMM can provide significant additional benefit in preventing clinical episodes of malaria as well as anaemia among children in Senegal.

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MALARIA DIAGNOSIS AND TREATMENT BEHAVIORS AMONG PUBLIC AND PRIVATE SECTOR HEALTH CARE PROVIDERS IN A PHASE IV TRIAL IN NORTHERN GHANA

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Globally, malaria control programmes are threatened by the development of drug resistance to monotherapies necessitating revision of treatment policies. Since artesunate-amodiaquine (ASAQ) replaced chloroquine as the first line treatment in 2002 in Ghana, little has been documented on the diagnosis and treatment behaviours of providers. The study explored this theme in the context of a Phase IV trial in the Kassena-Nankana District of Ghana that seeks to provide safety and effectiveness data on how antimalarials work when delivered outside trial conditions. In-depth interviews were conducted with 18 health care providers in both the public and private sectors and illness narrative interviews with 32 individuals who suffered malaria two weeks prior to the interview. All interviews were audiotaped, transcribed into English and imported into NVivo 8 for content analysis. The data suggests differences in diagnosis and treatment habits between public and private providers. Although both rely on clinical symptoms for the diagnosis of malaria, only public providers reported occasional use of rapid diagnostic tests (RDT) and microscopy for malaria. Public providers blamed non-use on chronic shortages of RDTs while private providers were unfamiliar with that type of diagnostic. Public providers officially stock and prescribe only ASAQ but some unofficially stock other antimalarials for patients who suffer ASAQ side effects. Private providers favor antimalarials other than ASAQ because of their low cost, easy dosing schedule, little or no side effects, and perceived efficacy. Vast disparities exist between recommended practices and actual practices in both sectors. Public providers more commonly adhere to recommended diagnostic and treatment practices, however improvement is necessary in both sectors. Standard protocols for guiding practice that have been introduced in the public sector should be better implemented in both sectors. The need for a regulatory framework to formalize drug distribution and use in both the public and private sectors is long overdue.

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A RANDOMIZED TRIAL OF A NEW FORMULATION OF ARTESUNATE MEFLOQUINE COMPARED TO ARTHEMETHER LUMEFANTRINE FOR THE TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN ADULTS IN SENEGAL

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WHO recommands the use of ACTs for the management of uncomplicated malaria cases. A new dosage of Artesunate/Mefloquine (Artequin™ Mepha LTD) with 25mg/kg of mefloquine has been developed. This new formulation is in accordance with WHO recommendation to avoid the development of *Plasmodium falciparum* mefloquine resistant strains. However, limited data are avalaible about its effectiveness and tolerability. We conducted a non inferiority trial to assess the effectiveness and tolerability of the new formulation of Artesunate/Mefloquine (AS/MF) compared to Artemether-Lumefantrine (AL) in the treatment of adults with uncomplicated malaria in Senegal An opened randomized trial was carried out in the central part of Senegal from September 2010 to January 2011, including adults and adolescent using WHO 2005 protocol for *in vivo* drug evaluation. Eligible patients were randomised to receiver either AL or AS/MF; a clinical and biological follow-up was done until day 28 for all included patients. 50% of them were followed until day 42. End points included the ACPR at J28, incidence of clinical and biological adverse event. In IIT analysis ACPR at day 28 in after PCR correction was 94.9% for the AS/MF group versus 96.7% for AL group (p=0.42). By PP analysis, ACPR at day 28 after PCR correction was 99.3% for AS/MF and 98.6% for AL (p=0.99). Similar results were found at day 42. The non inferiority of AS/MF was demonstrated both in IIT analysis and PP analysis at day 28 and day 42. Any clinical and biological severe adverse event was observed in the two groups. Our results show the good effectiveness, the good tolerance and the non inferiority of Artesunate-Mefloquine combination (Artequin™) dosed with 25mg/kg of mefloquine compared to Coartem®.

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A SYSTEMATIC REVIEW AND META-ANALYSIS OF NON-RANDOMIZED AND RANDOMIZED CONTROLLED STUDIES OF ARTESUNATE AND AMODIAQUINE FOR THE TREATMENT OF UNCOMPLICATED *FALCIPARUM* MALARIA IN AFRICA

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Artesunate+amodiaquine (AS+AQ) is the second most widely used artemisinin combination therapy (ACTs) for uncomplicated malaria. Published and unpublished non-comparative, comparative randomized and quasi-randomized trials conducted between 1999-2010 were identified via electronic and manual searches through MEDLINE, EMBASE, LILACS and CENTRAL. Standard methodologies were applied for selecting trials and assessing quality. Additional information was obtained from the investigators to allow analysing data by site when multiple sites were involved. Primary endpoints were PCR-adjusted and crude parasitological outcomes by Day 28 on the per-protocol dataset. Random effects models were used to aggregate estimates of randomized controlled trials. Of 83 potential studies identified, 59 comparative and non-comparative trials met inclusion criteria. 55 studies (49 comparative at 81 study sites, 6 non-comparative at 10 sites) enrolling 21,330 patients (8,055 on AS+AQ) in 25 (22 African) countries contributed to the Day 28 efficacy analysis. 53 trials specifically recruited children. Target drug doses were generally AS 12mg/kg + AQ 30mg/kg over three days. Crude Day 28 failure rates for AS+AQ varied widely (0%-80%). After genotyping failures averaged 7% (0-39%). Sensitivity analysis produced failure rates of 5.6-7.8%. Of the 49 studies (81 sites) comparing PCR-adjusted Day 28 failure rates, AS+AQ was significantly more effective than AQ (RR=0.27 [95%CI 0.20; 0.33], AS (RR=0.08 [-0.64; 0.79]), chloroquine (RR=0.05 [0.01;0.08]), SP (RR=0.28 [0.16; 0.40]), AQ+SP (RR=0.46 [0.34; 0.57]), chloroquine+SP (RR=0.17 [0.09; 0.25]), and AS+SP (RR=0.64 [0.41; 0.87]) but not significantly different from artemether+lumefantrine 6 doses (RR=1.46 [0.89; 2.06] and dihydroartemisinin+piperaquine (RR=2.20 [-0.12; 4.14]. Only one comparison each was available with artemether+lumefantrine 4 doses and AS+mefloquine. PCR-adjusted results were highly heterogeneous (except for dihydroartemisinin+piperaquine). This review is an updated inventory of available AS+AQ efficacy data. This study differs from the published Cochrane Reviews in that it considers the effect of site and all comparators and uses also non-comparative studies. AS+AQ met the WHO recommended minimum PCR adjusted efficacy of ≥ 90% in most but not all countries in Africa.

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PHARMACOKINETIC INTERACTIONS BETWEEN THE ANTIRETROVIRAL AGENT EFAVIRENZ AND THE ANTIMALARIAL ARTEMETHER-LUMEFANTRINE IN HEALTHY ADULTS

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Drug-drug interactions are common in patients infected with human immunodeficiency virus (HIV), including in the setting of co-infection with malaria. Artemether and lumefantrine (AL) (co-formulated as Coartem) both are metabolized by cytochrome p450 (CYP) 3A4 with artemether converted to active dihydroartemisinin (DHA). Efavirenz, an antiretroviral drug for HIV-1, is a CYP3A4 inducer. The purpose of this study is to investigate the effect of efavirenz on the pharmacokinetic (PK) disposition of artemether and lumefantrine. This study used a crossover open-label design. Those completing sample analysis were blinded to study details. Twelve healthy adult volunteers received 6 doses of AL (80/480 mg) twice daily. After a two-week washout period, all subjects received a 26-day course of efavirenz (600 mg once a day) to reach steady-state, then resumed AL administration for 3 days. Blood samples were drawn following the sixth dose of AL on day 4 and day 31.Non-compartment PK analysis with WinNonlin 5.2.1 was used to calculate PK parameters. Coadministration of efavirenz with AL led to significant decreases in DHA exposure, as measured by t1/2, Cmax, and the area under the plasma concentration versus time curve (AUC). Specifically, Cmax, AUClast, and AUC0-inf decreased by 39%, 46%, and 39% (p<0.05), respectively. Trends toward decreased exposure of artemether were noted during co-administration compared to AL administration alone (AUClast and AUC0-inf decreased by 51% and 34%, respectively). There was no statistical significance for the change in the DHA:artemether AUC ratio (p=0.824). For the long-acting partner drug, lumefantrine, a trend in decreased AUClast and AUC0-inf was noted (21% and 22%, respectively), although results did not reach statistical significance. Lastly, there was no significant effect of AL on efavirenz exposure. Decrease of artemether and lumefantrine exposure in the context of efavirenz deems to be modest in healthy HIV-negative adults and no dose adjustment is suggested.

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CLINICAL MANIFESTATIONS OF NEW VS. RECRUDESCENT MALARIA INFECTIONS IN AN EFFICACY STUDY

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The results of antimalarial drug efficacy studies undergo correction based on genotyping to exclude new infections that occur during the follow up period. This allows researchers to compare efficacy across differing transmission settings. However, censoring new infections may not be optimal for policymakers in developing countries. In the era of artemisinin-based combination therapy, highly effective but short-acting drugs may produce better "efficacy" than drugs with a prolonged post-treatment prophylactic effect. Further information is required to determine if new vs. recrudescent infections have different clinical implications for patients and if differential prevention of one of these types of infections will have greater public health benefit. We extracted DNA from dried filter papers collected from participants with recurrent parasitemia during drug efficacy studies of sulfadoxine-pyrimethamine in Malawi from 1998 through 2005. We used six neutral microsatellites to genotype the initial and recurrent infections and classified recurrent infections as new, recrudescent or indeterminate. Logistic regression and chi-squared analyses were used to compare the rates of fever and anemia, the clinical outcomes of interest. The recurrent infections were equally distributed between new and recrudescent episodes. The risk of fever and anemia were the same in new and recrudescent infections. The final analyses are being completed and precise risk estimates will be reported. Our study results suggest that both new and recrudescent infections should be considered to be treatment failures when countries select a drug for the national treatment policy. The prophylactic value of longer-acting drugs that reduce the risk of new infections should not be discounted in measurements of antimalarial drug efficacy.

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POPULATION PHARMACOKINETICS OF PIPERAQUINE IN CHILDREN AND ADULT PREGNANT AND NON-PREGNANT PATIENTS WITH UNCOMPLICATED MALARIA

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Malaria is one of the most important infectious diseases in the world. One of the most promising new artemisinin-based combination therapies is the fixed oral piperaquine and dihydroartemisinin combination. Children and pregnant women are especially vulnerable to malaria and the fetus is adversely affected. Reports describing food-interactions and pharmacokinetic properties of this combination in different patient populations are limited. Pharmacokinetic studies were conducted in Thailand (98 children and adults; 24 pregnant women and 24 non-pregnant women; 15 fed adults and 15 fasting adults), in Sudan (12 pregnant and 14 non-pregnant women) and in Burkina Faso (236 children). These studies investigated the pharmacokinetic properties of piperaquine after a standard oral three-day fixed dose regimen of dihydroartemisinin-piperaquine in patients with uncomplicated *falciparum* malaria. Dense venous or capillary plasma samples were collected and drug measurements conducted according to published methods. Concentration-time profiles were characterized using nonlinear mixed-effects modeling or non-compartmental analysis. The pharmacokinetic properties of piperaquine will be described using an individual and pooled analysis approach to investigate the impact of food and the pharmacokinetic differences in these populations.

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DETERMINANTS OF ACCESS TO ACTS AND MALARIA DIAGNOSIS: RESULTS FROM A HOUSEHOLD SURVEY IN THREE REGIONS OF TANZANIA

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While a general consensus over the choice of artemisinin based combination therapy (ACT) as the most effective malaria therapy has developed, a solid evidence-base for choosing the best ACT deployment strategies to gain optimal impact on malaria morbidity and mortality does not exist. Countries are now beginning to adopt policies to enhance ACT deployment that fall more or less into two basic paradigms: (i) making ACTs more readily and speedily accessible to patients, or (ii) targeting ACTs to patients shown to have malaria parasitemia. To design strategies to address and balance these goals, a detailed understanding of current treatment seeking patterns and their determinants is required. We therefore conducted large scale household surveys in three regions in Tanzania with varying transmission levels.5,429 households and 20,973 people were interviewed in Mbeya, Mtwara and Mwanza Regions between June and September 2010. All members of each household who were present and reported fever in the previous two weeks were asked about treatment sought, drugs obtained and the cost of this treatment. Additional data collected covered socio-economic status, net ownership and usage, and knowledge of malaria. Fingerprick blood samples were taken to test for malaria parasitaemia and for anaemia in children under five years. We will present results on the following two key outcomes: percent of people with fever who got an ACT (within 24 or 48 hours), and percent of people with fever who got a finger-prick or heel stick test. We will explore the determinants of these outcomes, considering the influence of age, socio-economic status, location, knowledge and treatment source. Finally, we will identify policy implications for strategies to improve ACT access and targeting, focusing on the current role out in Tanzania out of rapid diagnostic tests to public health facilities, and subsidised ACT under the Affordable Medicines Facility-Malaria.

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THROMBOCYTOPENIA IN MALARIA AND PROGNOSTIC UTILITY OF THROMBOCYTOPENIA IN *FALCIPARUM* MALARIA

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The incidence of thrombocytopenia in malaria is 67 - 90% according to various studies. Some studies suggest the possible role of platelets in the pathology of severe malaria while others have found a correlation of platelet count with prognosis. There are only a few studies done on this aspect in India. The aim of the study was to correlate the presence and severity of thrombocytopenia with type of malaria and to assess the utility of the initial platelet count as an independent prognostic marker for severe *falciparum* malaria. This is a prospective, observational study of patients > 18 years admitted in Medicine Department, in a tertiary

care teaching hospital from August, 2006 to July, 2008. Malaria was diagnosed based on clinical features along with positive Quantitative Buffy Coat method (QBC MP) or Thin Blood Smear examination (Giemsa stain). A total of 131 consecutive patients satisfying the diagnostic criteria during the study period were included. The data was then charted and analyzed using the SPSS 11.0 statistical software package for Windows. The Chi-Square test was used for comparative analysis of data of the different groups. Prevalence of thrombocytopenia was 88.3% and 88.6% in *vivax* and *falciparum* malaria respectively. Patients with severe *falciparum* malaria had a statistically significant lower platelet count (p value = 0.01) compared to non-severe *falciparum* malaria. In patients with severe *falciparum* malaria, those with renal failure (p = 0.019) and hyperparasitemia (p = 0.03) had a statistically significant lower mean platelet count compared to non-severe *falciparum* malaria. Patients with involvement of more than one organ system had a lower mean platelet count compared to those with single organ involvement. The conclusions were 1) *Vivax* malaria can also present with thrombocytopenia and complicated malaria. 2) The admission platelet count can be used to estimate the likelihood of complications and severity of malaria. Our study shows that admission platelet count is significantly lower in those who have hyperparasitemia and acute renal failure. 3) The platelet count is comparable between *vivax* malaria and non-severe *falciparum* malaria but significantly lesser in severe *falciparum* malaria.

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ESTIMATING THE NUMBER OF MALARIA INFECTIONS IN BLOOD SAMPLES USING HIGH-RESOLUTION GENOTYPING DATA

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People who live in malaria-endemic areas often harbour several infections at once. High-resolution genotyping can distinguish between infections by detecting the presence of different alleles at a polymorphic locus. However the number of infections may not be accurately counted since parasites from multiple infections may carry the same allele. We (i) propose a method to estimate the number of infections which would otherwise be detected, taking into account the probability of shared alleles and (ii) carry out simulations to determine the circumstances under which the number of infections is likely to be substantially underestimated due to multiple infections bearing the same allele. We use the allele frequencies to estimate the conditional probabilities of observing different numbers of genotypes given the true numbers of infections present. These probabilities are combined in a Bayesian model with the observed frequencies of genotypes and an assumed distribution for the numbers of infections. We evaluate this model using simulation and show that it can estimate the number of infections with reasonable accuracy. Simulations indicate that the problem is not substantial for most datasets. Large disparities between the number of infections and number of observed genotypes were limited to cases with fewer than 20 alleles, fewer than 20 blood samples, a mean number of infections of more than 6 or a frequency of the most common allele of more than 20%.

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IMPACT OF HEALTH FACILITY-BASED INSECTICIDE TREATED BEDNET DISTRIBUTION IN MALAWI: PROGRESS AND CHALLENGES TOWARDS ACHIEVING UNIVERSAL COVERAGE

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High levels of insecticide treated bednet (ITN) use can reduce malaria burden in countries with intense transmission such as Malawi. Since 2007 Malawi has implemented free health facility-based ITN distribution for pregnant women and children <5 years old (under-5s). We evaluated the progress of this targeted approach toward achieving universal ITN coverage. We conducted a cross-sectional household survey in eight districts in April 2009. We assessed household ITN possession, ITN use by all household members, and *P. falciparum* asexual parasitemia and anemia (hemoglobin <11 grams/deciliter) in under-5s. We surveyed 7,407 households containing 29,806 persons. Overall household ITN possession was moderate (59%) with 67% of eligible households (i.e. households with pregnant women or under-5s) owning an ITN and only 40% of ineligible households owning an ITN. ITN use in households who owned at least one ITN was high, with 76% of all household members, 88% of under-5s and 90% of pregnant women using an ITN the previous night. Of 6,116 ITNs, 92% were used the previous night with a mean of 2.4 persons sleeping under each ITN. In multivariable models adjusting for district, socioeconomic status and indoor residual spraying use, ITN use by under-5s was associated with a significant reduction in asexual parasitemia (adjusted odds ratio (aOR) 0.79, p-value 0.03) and anemia (aOR 0.79; p-value 0.04) prevalence. In addition, we explored potential targeted and non-targeted mass distribution campaign strategies as a means to achieve universal coverage. A campaign that distributes 1 ITN per household might achieve near universal coverage at 1.86 ITNs per household or 2.1 household members per ITN. In conclusion, Malawi has made substantial progress in ITN coverage using health facility-based distribution targeting pregnant women and under-5s, but will need to supplement these activities with non-targeted mass distribution campaigns to achieve universal coverage and maximum public health impact.

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RISK FACTORS FOR *PLASMODIUM FALCIPARUM* MALARIA ACQUISITION ABROAD BY UK RESIDENTS IN 2007

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An increasing proportion of malaria cases in UK residents which were acquired in malaria endemic areas are due to *Plasmodium falciparum*, the most virulent form of malaria, resulting in a number of hospitalisations and a small number of deaths (5-16 annually since 1991). Identifying the main risk groups is necessary to design appropriate public health strategies for reducing the number of cases. However, previous studies have found it difficult to account for exposure within malaria endemic areas. Here we estimate the entomological inoculation rate (infectious bites per person per day) to which travellers are exposed and their duration of stay in malaria endemic areas to calculate the probability of acquiring malaria in each country. We then estimate this risk for acquisition of malaria amongst travellers with different purposes of travel and in different age groups to identify high risk groups. A proportional hazards model was fitted to data

on the trips made by cases and the population as a whole, adjusting for the baseline risk in each country and the duration of stay. Both reason for travel and age-group were found to be significant determinants of the risk of acquiring malaria (p<0.0001). Those visiting friends and family and business travellers were at significantly increased risk of acquiring malaria (adjusted hazard ratio (HR) relative to that of holiday makers 6.0, 95% CI 3.5-11.8, p<0.01 and HR 2.3, 95% CI 1.5-7.6, p<0.01, respectively). All age-groups were at lower risk than children aged 0-15 years old (p<0.01). Travellers visiting friends and family, business travellers and young children remain at increased risk of malaria acquisition after adjusting for the risk of infection in their destination and their duration of stay. These groups should be the target of programmes to increase awareness of malaria acquisition when travelling.

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MOLECULAR EPIDEMIOLOGY OF *PLASMODIUM* INFECTIONS IN TWO MALARIA ELIMINATION SETTINGS IN VANUATU AND SOLOMON ISLANDS

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Malaria prevalence in Tanna Island, Vanuatu and Temotu Province, Solomon Islands has declined significantly due to effective malaria control programs and both have a commitment to provincial malaria elimination. As the first step toward elimination, mass blood surveys were conducted to obtain baseline epidemiology information. From these surveys, microsatellite genotyping was used to assess the number of parasite haplotypes being transmitted, their frequency, diversity and distribution. For *Plasmodium vivax*, genotyping revealed 22 haplotypes of the 75 isolates typed for Tanna, and 46 haplotypes of the 82 isolates typed for Temotu. The number of *P. vivax* haplotypes was approximately 4 times greater than that of the sympatric *P. falciparum* populations. In Tanna, while most *P. vivax* haplotypes were scattered on the island, some appear to be clustered to small regions indicating some limitation in human movement. In Temotu, no clustering was observed. Parasite population structure for both locations were analysed. The results provide good epidemiological data for both locations, and may reflect the malaria transmission levels. These data can be used to monitor any change in malaria epidemiology and transmission, as well as progression toward elimination.

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MOLECULAR EPIDEMIOLOGY OF MALARIA IN SOUTHEASTERN BANGLADESH, WITH THE MAIN FOCUS ON THE SYMPATRIC DISTRIBUTION AND DIAGNOSIS OF *PLASMODIUM OVALE WALLIKERI* AND *P. OVALE CURTISI*

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In spite of the high prevalence of malaria in Southeastern Bangladesh, there remains a significant shortage of information regarding the presence of four out of six human malaria parasites: *Plasmodium ovale wallikeri, P. ovale curtisi, P. malariae* and *P. knowlesi.* The molecular epidemiology of these pathogens as well as of the more prevalent species *P. falciparum* and *P. vivax*, were investigated within the course of field surveys and a hospital-based survey in Bandarban District in Southeastern Bangladesh between 2006 and 2010. Filter paper samples from 1,867 asymptomatic participants and 379 patients presenting with symptomatic febrile illnesses were analyzed using a genus- and species specific nested PCR method, targeting the small subunit ribosomal RNA (SSU rRNA) gene. Samples positive for *P. ovale* spp. were further analyzed by multilocus sequence analysis of 3 loci (cox1, SSU rRNA, porbp2) and the comparison of several different PCR techniques targeting the SSU rRNA and PoTRA genes for their accuracy regarding the diagnosis of *P. ovale* spp. In the course of this study a new PCR method was established and tested for its accurate diagnosis of *P. ovale*. In both, symptomatic and asymptomatic participants, *P. falciparum* was the dominant species, followed by *P. vivax* and the less prevalent parasites *P. malariae* and *P. ovale* spp. We found a high rate of mixed infections and asymptomatic malaria cases in this region. However, there was no indication of the presence of *P. knowlesi* in Southeastern Bangladesh. Our data provide the first evidence of *P. ovale wallikeri* and *P. ovale curtisi* in Bangladesh and their sympatric distribution in South Asia.

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THE DISTRIBUTION OF HUMAN *PLASMODIUM* SPECIES IN CENTRAL VIETNAM IS COMPLEX WITH MARKED AGE-DEPENDENT PREVALENCE OF SYMPTOMATIC AND PATENT INFECTIONS

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In Vietnam, *Plasmodium falciparum* and *P. vivax* are responsible for the majority of malaria infections while *Plasmodium malariae* and *P. ovale* infections are rarely reported. Nevertheless, a species specific PCR analysis on 2,303 blood samples, collected during a cross sectional survey carried out in a forest area of malaria endemic province in central Vietnam, identified 223 (prevalence = 9.7%) *P. falciparum*, 170 (7.4%) *P. vivax*, 95 (4.1%) *P. malariae*, and 19 (0.8%), *P. ovale* mono-infections with mixed infections occurred at and 164 (7.1%) mixed infections. Out of the 671 positive samples positive by PCR (prevalence sub-patent infections=29%), only 331 (50%) were positive also by microscopy. *P. malariae*, *P. ovale* and mixed infections were poorly diagnosed by microscopy. Although, clinical and sub-clinical infections occurred in all age groups, the risk of infection and disease decreased significantly with increasing age. The parasite densities and the prevalence of patent infections were significantly lower in the adult population, probably due to the acquired partial immunity. The common occurrence of sub-patent infections seems to indicate that the malaria burden is largely underestimated which calls for the urgent development of improved diagnostic and surveillance tools for future elimination perspectives and that diagnostic and therapeutic policies should be adapted accordingly.

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ESTIMATES OF THE IMMUNE PARAMETERS DETERMINING THE DURATION OF ANTIBODY RESPONSE TO *PLASMODIUM FALCIPARUM* INFECTION IN GHANAIAN INFANTS

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Infants in regions of intense malaria transmission are particularly vulnerable to malaria in their first years of life before they have acquired substantial immunity. Understanding the processes underlying the acquisition and loss of immune effector mechanisms is crucial for understanding the epidemiology of the disease and the likely impact of vaccines. From a longitudinal study of 151 Ghanaian infants followed for over 2 years we fitted biological models of immunological processes to IgG antibody titres to five *Plasmodium falciparum* antigens using nonlinear mixed effects models to investigate the acquisition and loss of humoral

immune responses. We assume that exposure to *P. falciparum* antigen induces proliferation of antigen-specific B cells and their differentiation into IgG antibody secreting plasma cells and that the duration of the IgG response is dependent upon (i) the half life of individual IgG molecules, (ii) the half life of IgG secreting cells and (iii) rates of reinfection which induce differentiation of new populations of memory B cells and IgG secreting cells. For apical membrane antigen 1 (AMA1) we estimate that maternally-acquired antibodies have a half-life of 72 days (95% CrI 64-82); exposure induced antibodies have a half-life of 152 days (95% CrI 126-186); and ~2% of antigen-specific B cells differentiate into long-lived memory B cells. For merozoite surface protein 1 (MSP1) we estimate that maternally-acquired antibodies have a half-life of 64 days (95% CrI 55-75); exposure induced antibodies a half-life of 57 days (95% CrI 49-79); and ~1% of antigen-specific B cells differentiate into long-lived memory B cells. These results demonstrate that whilst circulating antibodies decay over a period of weeks as measured in other studies, antigen-specific memory B-cells may circulate for months to years providing a degree of immunity to re-infection.

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THE IMPORTANCE OF CELL PHONE IMPLICATION AS A DYNAMIC TOOL TO GET MALARIA POSITIVE CASES AT REMOTE KUHALONG AND RAJBILA UNIONS IN BANDARBAN DISTRICT, BANGLADESH

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The tribal rural Chittagong Hill Tracts of Bangladesh is where malaria has highest prevalence rate and 1.5 million people are at risk. The ICDDR, B in collaboration with Johns Hopkins Bloomberg School of Public Health is conducting a malaria epidemiological study in two adjacent unions comprising a population of 20,000 individuals in an area over 172 square kilometres. About 20% of the communities are densely forested and have no road access. The recent introduction of cell phones two years ago in the rural areas of Bandarban district has opened new doors to using this technology for epidemiological purposes as more than one third (1401) households in this region own at least one cell phone or are able to borrow one. The study subjects have been instructed to phone the study physician with fever and associated symptoms of malaria. Villagers know that they will receive free medicine and latest RDTs to identify malaria. From June 2010 until May 2011, there were 211 phone calls for potential malaria cases. Of the calls, a total of 54 were malaria-positive cases, which represents 27% of all 201 malaria-positive cases in the study unions since June 2010. In addition, a severe malaria patient in a remote area was saved because of the cell phone initiated the 2.5 hrs drive to a health facility. Costing only a few taka (cents) per call, represents a significant portion of the total number of positive cases, highlighting the importance of cell phones in this type of epidemiological activity. This method of immediate medical attention to malaria-positive patients is absolutely critical when considering strategies for malaria eradication in Bangladesh, and should be applied to other malaria endemic districts if the resources are available. Using current cell phone technology in the medical and scientific realms is effective, efficient, and should entice phone companies to broaden their coverage to all rural areas of the country.

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MAPPING MALARIA RISK IN CÔTE D'IVOIRE

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In Côte d'Ivoire, an estimated 767,000 disability-adjusted life years are lost annually due to malaria the disease remains of great public health importance, ranking the country at position no. 14 with regard to the global burden of malaria. The purpose of this study was to predict malaria infection risk in Côte d'Ivoire for children aged <16 years. A geostatistical modeling approach was employed using point-prevalence data from published and unpublished work. Four Bayesian regression models, two of which were spatially-explicit, were fitted for *Plasmodium* spp. infection prevalence using a suite of environmental covariates (i.e. elevation, distance to water bodies, maximum land surface temperature and rainfall). Model fits were compared with the deviance information criterion. A total of 235 georeferenced *Plasmodium* prevalence data points from surveys including children aged <16 years were obtained from published and unpublished work carried out between 1988 and 2007. The majority of data points (n=182, 64 %) were collected between 2000 and 2007, whereas the remaining 53 data points were obtained from surveys between 1988 and 1999. The best fitting model was a Bayesian non-stationary regression model, with rainfall and land surface temperature identified as significant covariates. This model was used for prediction and mapping of *Plasmodium* spp. infection risk at non-sampled locations. The obtained malaria risk map can be utilized for spatial targeting of control interventions, which is important for the national malaria control program in Côte d'Ivoire.

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MALARIA ATTRIBUTABLE FRACTION OF FEVER ACCORDING TO SEASON IN A MALARIA VACCINE TRIAL SITE OF BURKINA FASO

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In Burkina Faso, malaria management policy recommends presumptive treatment of malaria in children under five years presenting at the peripheral health facility with fever. This policy facilitates early management of malaria, but could also delay the care of other etiologies of fever such as septicemia and increased risk of child mortality. This study aims to estimate the malaria attributable fraction of fever in a vaccine trial site of Burkina Faso. We conducted two community-based cross-sectional surveys in children aged 0 months to 5 years of age from four villages of the health district of Saponé. The first survey was conducted during the rainy season and the second in the dry season. Parasitological and clinical examinations were performed. A fever case was defined as objective temperature ≥37.5 °C or history of fever in the past 24 hours. Fever was more prevalent during the rainy season (91/487; 18.7%) than the dry season (73/522; 14.0%). The malaria attributable fraction of fever presented the same trend with 41.9% during the rainy season and 34.5% during the dry season. The alternative parasite thresholds for the malaria case definition that achieved optimal sensitivity and specificity (70-80%) were 1350 parasites/µl during the low season and 3150 parasites/µl during the high season. Our results confirm that malaria is a main cause of fever in the Saponé health district. The relationship between fever and parasitaemia depends on the season. Burden of the disease is

higher during the malaria high transmission season. Malaria management policy should recommend the use of microscopic or at least RDT, before administration of anti-malarial drugs during the dry season.

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MARKERS OF INFECTION AND EXPOSURE TO MALARIA IN AFGHAN REFUGEE CAMPS IN KHYBER PUKHTOON-KHWA (KPK) PAKISTAN

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In South Asia, Pakistan is amongst the most malarious countries and large-scale epidemics have been reported in the past. Khyber Pukhtoon-Khwa (KPK), Northern Pakistan is characterised by seasonal transmission with predominantly *Plasmodium vivax* malaria. The high number of Afghan Refugees in KPK has compounded the malaria problem as the malaria incidence in this group is relatively high. Therefore this province needs an effective and sustainable programme to achieve the overall objectives of malaria control. The project investigated the use of sero-prevalence to measure transmission intensity and to identify the risk factors in KPK. The study may provide additional insight into historical patterns of malaria transmission, the dynamics and kinetics of immunity in the population. KPK is low endemic area and suitable for malaria elimination, but assessing transmission is difficult because of lack of sensitivity of commonly used methods. We used serologic markers to detect variation in malaria exposure in these refugee camps. This information will help to guide researchers and decision-makers in targeting intervention efforts. A cross-sectional survey was conducted in five Afghan refugee camps of Khyber Pukhtoon-Khwa (KPK), Northern Pakistan between June and September in 2010. Blood samples were obtained on filter paper from three household members to measure parasite prevalence, transmission intensity and exposure using serology (ELISA). Infection status was determined by Rapid Diagnostic Test (RDT).Sub-samples are also assayed using molecular techniques (PCR) to identify low density parasite infections. PCR results will allow serological data for examination of the effect of parasite carriage on seropositivity and evaluation of RDT. Data will be presented on age sero-prevelance of *Plasmodium falciparum* and *Plasmodium vivax* merozoites surface proteins ($MSP1_{19}$). Parasite rate will be assessed by RDT and PCR for both *Plasmodium falciparum* and *vivax*. Results will provide an up to date insight in to the region's malaria transmission intensity.

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EFFECT OF MALARIA TRANSMISSION SEASON ON HEMATOLOGIC MEASUREMENTS IN HEALTHY MALIAN CHILDREN LIVING IN A MALARIA ENDEMIC AREA

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Hematological indices are commonly used in epidemiological studies of malaria: e.g., use hemoglobin (Hb) values to define anemia, calculate parasitemia based on the number of white blood cell (WBC) on a slide, etc. However, few studies report the effects of malaria transmission season on the hematological measurements. Therefore, we collected venous blood from 249 Malian children aged 3-12 years living in a village where malaria transmission is seasonal. Red blood cell (RBC) count, Hb, hematocrit (Ht), WBC count, and WBC subsets were determined in May 2010 (beginning of the transmission season) and January 2011 (end of the season). At the time of blood collections, all of the children appeared to be healthy, and none of children in May and 19% children in January were determined as *Plasmodium falciparum* positive microscopically. At the beginning of the season, older children showed higher median Hb levels (10.9 g/dl for 3-5 years vs. 11.8 for 9-12 y, $p<0.001$) and Ht (36% for 3-5 y vs. 38.1 for 9-12 y, $p<0.001$), while RBC counts were the same regardless of age. On the other hand, older children showed lower WBC counts (7.81 x 10^3/ml for 3-5y vs. 5.72 x 10^3 for 9-12 y, $p<0.001$). The children experienced an average of 1.1 malaria episodes during the transmission season. From May 2010 to January 2011, the children showed higher levels of RBC (4.51 to 4.68 x 10^6/ml, $p<0.001$), Hb (11.3 to 11.8 g/dl, $p<0.001$) and Ht (36.9 to 39.0 %, $p<0.001$), while the level of WBC was lower in January (6.62 to 5.96 x 10^3/ml, $p<0.001$). There were no correlations between the changes in the hematological indices and the number of malaria episodes experienced during the transmission season. Relative numbers of WBC subsets also changed from May 2010 to January 2011; 34 to 42 % neutrophils, 6.6 to 7.7 % monocytes and 54 to 45 % lymphocytes ($p<0.001$ for all of comparisons). Although our study did not uncover the mechanisms responsible for the changes, the results suggest that it is inappropriate to assume that hematological values are constant regardless of age and/or season.

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PARTURIENT KENYAN WOMEN ACCURATELY SELF-REPORT MALARIA AND BENEFIT FROM SELF-REPORTED USE OF SULFADOXINE-PYRIMETHAMINE BUT ARE ALSO AT RISK FOR HIGH DENSITY PLACENTAL INFECTION

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Using a large cross-sectional study (n=1,082) conducted with mothers at parturition in western Kenya, associations between self-reporting of malaria (SRMal) and clinical diagnosis of placental malaria (PM), and between medication use, specifically sulphadoxine-pyrimethamine (SP), and maternal and fetal outcomes were investigated. A strong correlation was found between SRMal and microscopic diagnosis of PM (OR: 2.69 CI: 1.57-4.60). Although SP users had a reduced risk overall for PM (OR: 0.60 CI: 0.35-1.02) and had longer gestations (OR: 1.52 CI: 1.11-2.09), those SP users who had PM were more likely to have high levels of parasitemia (OR: 3.87 CI: 1.33-11.27). The results show SRMal in areas holoendemic for malaria can be used to inform decisions about treatment of pregnant women when resources are limited and laboratory diagnosis is unavailable. Furthermore, while SP appears efficacious in protecting against PM and poor birth outcomes, drug failure may contribute to risk for high density placental parasitemia.

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SINGLE DOSE MASS DRUG ADMINISTRATION OF AZITHROMYCIN DECREASES MALARIA INCIDENCE IN A LARGE COHORT TREATED FOR OCULAR TRACHOMA

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Single dose mass drug administration of azithromycin (AZ MDA) reduces community wide trachoma rates and all-cause mortality. We investigated the malaria protection, and selection for *Plasmodium falciparum* resistance following a trachoma intervention that included single dose AZ MDA.

A cohort of 1,086 participants from AZ MDA treated villages and 1,063 controls was drawn from 8 rural villages in central Tanzania and followed from January through July 2009. Participants from 4 treated villages received single dose azithromycin (20 mg/kg in children, 1 g in adults). Blood samples were taken on filter paper from the participants at baseline, and months 1, 3, 4, and 6. Diagnosis for *P. falciparum* infection was made by detection of 18S ribosomal DNA in real-time quantitative PCR. Data were analyzed with a multivariate logistic regression controlling for self-reported bed net ownership, anti-malarial use, rainfall, and altitude. Random effects were used to account for clustering within villages. In the first month after AZ MDA, 13 of 796 (1.63%) participants from AZ MDA villages tested positive for *P. falciparum* compared to 34 of 739 (4.40%) participants in control villages. The odds of incident *P. falciparum* infection decreased 67% (95% CI: 39%, 82%) in AZ MDA treated villages compared to controls in the first month after treatment; however in the ensuing months AZ MDA control and treated villages had similar proportions of infection. Sequencing of the *P. falciparum* ribosomal L4 protein from more than 50 clones from a dozen patients indicates no changes consistent with drug resistance to azithromycin. These data provide evidence that AZ MDA retains a transient anti-malarial prophylactic effect, and that AZ MDA has not selected for resistance mutations to the ribosomal L4 protein target in a region with a ten-year history of azithromycin use for trachoma.

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BASIC EFFICACY OF ORAL INSECTICIDES IN TOXIC SUGAR BAITS TO CONTROL MOSQUITOES AND SAND FLIES IN THE LABORATORY

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The use of oral insecticides in sugar solution is a promising method that requires good basic oral efficacy against target species. A series of feeding experiments in the laboratory was performed to test the palatability of toxic sugar baits (TSB) and the basic efficacy of oral insecticides (spinosad, thiamethoxam, dinotefuran, boric acid) in TSB against male and female sand flies (field-collected *Phlebotomus sergenti* and laboratory-reared *P. papatasi*) and mosquitoes (*Culex pipiens, Anopheles stephensi* and *Aedes aegypti*) exposed to a series of insecticide dilutions for 24 h. Cumulative mortality rates were determined at 24, 48 and 72h post-exposure, and LC values were calculated. The persistence of TSB was tested in field conditions in Israel. Flies fed on all types of baits tested, and neither insecticide deterred feeding. Mortality was dose-dependent and faster on TSB with thiamethoxam or dinotefuran than TSB with spinosad or boric acid. Feeding and mortality rates differed between old and young age cohorts. Residual persistence of TSB highlighted important differences between the insecticides. Their suitability for potential use in TSB for vector control will be discussed.

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HOW SHOULD NOVEL VECTOR CONTROL TOOLS BE TESTED?

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Long lasting insecticidal nets (LLINs) were developed and recommended by the World Health Organization in the early 2000s, which enabled the massive scale-up of vector control that has been seen in recent years. Today, insecticidal tools form the basis of vector control interventions in many disease control initiatives, including the Global Malaria Action Plan that strives for Universal Coverage of at-risk populations using locally appropriate tools such as LLINs or indoor spraying of residual insecticides. To date, pyrethroids are the only class of insecticide approved by the WHO for use on mosquito nets, for reasons of safety, efficacy, acceptability and cost. The development of insecticide resistance in mosquito vectors is of increasing concern and evidence on the extent of the resistance problem is mounting. Reports of reduced efficacy of pyrethroid-treated nets in several countries with documented insecticide resistance underscore the need for new paradigms in vector control. Much of the research on the efficacy of non-pyrethroid insecticides on nets has been performed in experimental huts, which can be used to compare one product with another at a certain point in time and space. However there is very limited published information relating results from experimental hut trials to the effectiveness of vector control tools at village level. New vector control interventions will differ greatly in their mode of action, efficacy, features and applicability in different settings but new and specific categories for novel disease control tools are required. Clear testing guidelines for new categories of interventions will help to drive innovation by clearly defining the product requirements and a providing a clear pathway to a market that will recognise the value of those novel products.

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ESTABLISHMENT OF AN INSECTICIDE RESISTANT COLONY OF *ANOPHELES GAMBIAE* FOR THE ASSESSMENT OF NEW VECTOR CONTROL TOOLS

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Vector control today is heavily dependent on insecticide-based interventions, many of which are based on pyrethroids for example insecticidal nets, indoor residual house spraying and new products such as durable wall lining. Current product evaluation guidelines rely on standard susceptible laboratory strains, such as the KISUMU strain of *Anopheles gambiae* for efficacy testing of interventions. However many countries are now reporting insecticide resistance resulting in an increasing need to test new vector control tools in the laboratory using a 'standard' resistant mosquito strain. A field collected strain of *An. gambiae*, resistant to the main insecticides used in public health and agriculture (e.g. pyrethroids, organochlorines, carbamates and organophosphates) from Tiassalé village in Ivory Coast was colonized and maintained in the laboratory over 12 generations. The strain was fully characterized using the WHO susceptibility test, Hot Oligonucleotide Ligation Assay (HOLA) for East- and West-African *kdr* mutation assessment and an indirect determination of metabolic resistance using bioassays with a range of synergists. Mortality rates from WHO susceptibility tests revealed a strong level of resistance to pyrethroids (deltamethrin = 65.33%, permethrin = 45.58%), organochlorines (DDT = 4.10%), organophosphates (fenitrothion = 86.48%) and carbamates (bendiocarb = 33.82%, propoxur = 13.88%). After 12 generations in the lab without selection, the resistance levels to these compounds were higher than at the beginning (deltamethrin = 17.50%, permethrin = 2.50%), organochlorines (DDT = 2.63%), organophosphates (fenitrothion = 54.43%) and carbamates (bendiocarb = 0%, propoxur = 2.59%).The various insecticide resistance mechanisms investigated included target site resistance (*kdr* mutation) and metabolic resistance and will be discussed in the context of how this strain can be used for estimating the efficacy of new tools.

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THE "AUTO-DISSEMINATION" APPROACH: A NOVEL CONCEPT TO FIGHT *AEDES ALBOPICTUS* IN URBAN AREAS IN ITALY

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In the last 20 years, *Aedes albopictus* has become a permanent pest and an intractable nuisance problem in urban and peri-urban areas of most Italian regions. In the last year, the species has also colonized other parts of Europe and now presents a significant public health risk, as shown by the 2007 Chikungunya outbreak in Emilia Romagna (Italy). Most municipalities in Northern and Central Italy, where *Ae. albopictus* reaches its highest densities, carry out expensive control campaigns which aim to decrease larval (and thereby adult) densities by treating urban drainage systems with larvicides. The success of these interventions is hampered by the sheer number of alternative breeding sites available and by *Ae. albopictus*' propensity to subdivide their progenies among different unstable aquatic habitats in order to maximise their chances of successful development (skip oviposition). The difficulty in targeting these habitats is also increased by the significant presence of small and medium sized water containers in private gardens, courtyards and terraces. We will present the results of laboratory and field experiments carried out in Rome to test whether the "auto-dissemination" approach successfully exploited against *Ae. aegypti* in Peru (reported previously) could be applied to interfere with the development of *Ae. albopictus* larvae in a n Italian setting as well. This method exploits wild adult mosquitoes to disseminate a juvenile hormone analogue between contaminated resting sites and oviposition sites. The distribution of the hormone in the mosquito breeding sites is therefore very accurately and efficiently targeted. Moreover, the technique is interesting because of the amplification in coverage seen between resting and oviposition sites. Results showed significant larval mortality in sentinel versus control larval sites, confirming that the "auto-dissemination" approach has a very good potential as a novel control strategy, and that it may allow unparalleled coverage of aquatic *Ae. albopictus* habitats in urban areas of Italy, as well as of other countries.

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CO-DEPLOYMENT OF WHO ASSAY AND CDC BOTTLE ASSAY FOR INSECTICIDE RESISTANCE SURVEILLANCE IN ZAMBIA

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Insecticide resistance is determined primarily by one of two roughly equivalent *in vivo* assays the "WHO tube assay" and the "CDC bottle assay". Both are limited by availability of mosquito specimens and the skills needed to conduct the tests and interpret the results. The WHO assay is widely employed but its cost, storage and shipment requirements pose limitations for use in remote endemic areas. A study to evaluate the possible emergence of insecticide resistance in malaria vectors *Anopheles gambiae* s.s to two classes of insecticides, Organo Chlorines and pyrethroids, was conducted in Mushili-Commando and Chipulukusu compounds of Ndola District between 2009 and 2010. The study was conducted using the established CDC bottle assay protocol and presented evidence of resistance to DDT, permethrin, deltamethrin, and suspected resistance to lambdacyhalothrin and alpha-cypermethrin. The findings where validated by using the WHO tube assays. Both assays showed high levels of DDT and permethrin resistance. Mortality at diagnostic time was between 3% and 17%, and 8% and 51% for DDT and permethrin respectively. The standard WHO discriminating dosages showed 23.7% and 43% mortality for DDT and permethrin respectively. The emerging problem of insecticide resistance in Zambia threatens the future effectiveness of indoor residual spraying (IRS) and Long Lasting Insecticidal nets (LLITNs), and necessitates intensive resistance surveillance. For sustainable establishment of robust resistance monitoring in operational research to strengthen malaria control and elimination efforts, simple and affordable methods, with parsimonious reagent and equipment requirements are essential. To maximize the operational monitoring of insecticide resistance in vector populations, Zambia has adopted the use of both assays in spatially segregated areas, the CDC bottle assay for routine monitoring in rural remote areas while WHO assays are utilized in areas with ease of access to entomological laboratories and are employed for validation of CDC bottle assay results precedent to policy decision making.

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INSECTICIDE SUSCEPTIBILITY STATUS OF *ANOPHELES GAMBIAE S.L.* IN NORTHERN UGANDA

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Larger scale chemical vector control programs in Africa working on malaria prevention and control have had a growing concern regarding the development and spread of malaria vector resistance to public health insecticides. A study conducted in six districts of Northern Uganda in 2010, collected vector-insecticide resistance data to inform decision making about which insecticide would be unaffected by existing vector-insecticide resistance patterns in the area. *Anopheles gambiae s.l.* were collected from each district and tested using a WHO insecticide susceptibility test. The insecticides tested included Carbamate (Bendiocarb), which killed 100% (n= 600) of the vectors tested, a result suggesting 100% susceptibility in all districts. Pyrethroid (Alpha-cypermethrin) caused 100% mortality (n= 400) in vector collections from four districts (Amuro, Apac, Oyam and Gulu) and a reduced susceptibility of 74.5% (n=420) in the remaining two districts (Kitgum and Pader). This study also confirmed reduced susceptibility with 30% mortality to DDT (n=460) in all districts. During following year, Organo Phosphate (Pirimiphos-methyl) and Bendiocarb were tested instead of Alpha-cypermethrin and DDT. Both insecticides produced 100% mortality (n=100 each) in all districts. These results indicate high levels of DDT resistance and reduced susceptibility to Alpha-cypermethrin in the target vector population and argue for the use of both classes of insecticides for malaria control in the region. The alternative, rotational or mosaic use of Bendiocarb and Pirimiphos-methyl in vector control programs could mitigate the likelihood of insecticide resistance in future. This study demonstrates the utility of information from routinely monitored vector-insecticide susceptibility tests to identify candidate insecticides for future vector control programs and to design resistance management strategies for IRS, LLITNs and other chemical vector control programs in the region.

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PLASTICITY AND HERITABILITY OF *IN VITRO* SPATIAL REPELLENCY BEHAVIORAL RESPONSES IN *AEDES AEGYPTI* MOSQUITOES

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There is currently much interest in the potential to develop novel vector control interventions based on spatial repellency (SR) to combat disease transmission at sites that are unaffected by traditional tools such as indoor residual spraying (IRS) and bed nets (ITNs). Despite the fact that behavioral modification as a means to disease reduction has been recognized for

more than 60 years, the underlying mechanisms of SR behavior remain poorly understood. Of particular relevance to a field-based intervention intended to exploit SR is the need to describe the behavioral plasticity and heritability of SR in the target vector(s) through generations - will there be selection for responders over time? We present results on 1) the reproducibility of behavioral responses observed in individual cohorts of mosquitoes exhibiting both positive (responders) and negative (non-responders) SR behavior and 2) selective breeding experiments designed to illustrate the heritability of SR based on the proportion of SR responders in subsequent mosquito cohort generations. Studies were performed under laboratory conditions against *Aedes aegypti* (THAI strain) mosquitoes exposed to varying spatial repellent products.

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REDUCED SUSCEPTIBILITY TO PYRETHROIDS IN *ANOPHELES GAMBIAE* POPULATIONS OF WESTERN KENYA

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As malaria control interventions directed against *Anopheles* vectors increase in sub-Saharan Africa, it is crucial to assess the sensitivity of the mosquito vectors to the insecticides used in the programs. The use of ITNs is widespread in western Kenya with up-scaling of IRS in targeted districts. Therefore, we investigated the susceptibility of *An. gambiae* and *An. arabiensis* to pyrethroid and carbamate insecticides. Adults for analysis were reared from field-collected larvae or from eggs of wild-caught females, from Ahero, Budalangi, and Bungoma. Phenotypic resistance was determined using WHO test kits and time-dependent, bottle bioassays. Microplate enzyme assays were done with insecticide-exposed and non-exposed mosquitoes to investigate whether detoxifying enzymes were over-expressed compared to controls. The frequency of knockdown resistance (KDR) alleles was determined by RT-PCR while species were identified using standard PCR in 3,396 mosquitoes. The Bungoma sample was comprised of 74% *An. gambiae* s.s. and 26% *An. arabiensis* (n=904). About 99.4% of the *An. gambiae* s.s. were homozygous for the KDR genotype while the allele was absent in *An. arabiensis* (n=251). *An. gambiae* s.s. populations from Bungoma showed phenotypic resistance to permethrin (62%), deltamethrin (34%) and moderate resistance to bendiocarb (12%). Samples from Budalangi were also moderately resistant to permethrin (26%) and had slightly reduced sensitivity to deltamethrin (15%). There was no significant difference between the CDC Bottle and WHO tube bioassay methods suggesting that either method can be used to accurately quantify insecticide resistance. *An. arabiensis* were susceptible to all the three insecticides and none had any of the KDR genotypes. Permethrin resistant *An. gambiae* s.s. from Bungoma 1.8 fold elevation in nonspecific esterases most likely due to the high frequency of organophosphate insecticides used in the area and thus may be responsible for insecticide resistance in synergy with kdr.

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MOLECULAR MECHANISMS OF PYRETHROID RESISTANCE IN FIELD POPULATIONS OF *ANOPHELES FUNESTUS*, MAJOR MALARIA VECTOR IN AFRICA

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Although more cases of insecticide resistance are being reported in field populations of *Anopheles funestus* in Africa, the underlying molecular mechanisms remained uncharacterised contrary to the other major malaria vector *An. gambiae*. To fill this gap in our knowledge, we have been investigating mechanisms of pyrethroid resistance in field populations of this species from different regions of Africa. Different resistance patterns have been observed between *An. funestus* populations from different regions in Africa. Pyrethroids/carbamate resistance is observed in Southern Africa, Pyrethroids/DDT in East, Pyrethroid/Carbamate/DDT in West and DDT/Dieldrin in Central Africa. To generate new genomic tools to investigate mechanisms of resistance in this species, the transcriptome of *An. funestus* was sequenced using 454 pyrosequencing and generated 18,000 ESTs used to design a whole genome microarray chip. By comparing pyrethroid resistant field populations to susceptible samples in microarray analysis, we identified that resistance is mainly conferred by two duplicated P450 genes CYP6P9a and CYP6P9b with varying involvement depending of the country. Other detoxification genes such as CYP6Z1, CYP6P4a/b plus others genes seems also to be playing a role in the resistance. This microarray result was confirmed by qPCR. RNA interference confirmed the involvement of CYP6P9a/b in the resistance. CYP6P9a/b were also shown to metabolise pyrethroid *In vitro* using a recombinant enzyme of each copy. Polymorphism analysis between resistant and susceptible identifies significant differences and analysis is currently carried out to determine the causative ones. Analysis of haplotypes of the voltage gated sodium gene associated with the knockdown target site resistance indicated a correlation with pyrethroid resistance but the common L1014F mutation was not identified after screening samples from 7 countries. The characterization of these resistance mechanisms in *An. funestus* will help to improve the implementation and management of future malaria vector control programs in Africa.

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AN IMPROVED AUTOCIDAL GRAVID OVITRAP (CDC-AGO) FOR THE CONTROL AND SURVEILLANCE OF *AEDES AEGYPTI*

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Limited success has been achieved using traditional vector control methods to prevent the transmission of dengue viruses. Integrated control programs incorporating alternative tools, such as gravid ovitraps (lethal ovitraps, sticky ovitraps) may provide a greater potential for reducing vector populations and dengue transmission. We developed the CDC autocidal gravid ovitrap (CDC-AGO) as a simple, low-cost device for surveillance and control of *Aedes aegypti* without the use of pesticides that does not require servicing for an extended period of time. In a previous area-wide, intervention study in southern Puerto Rico, it was estimated that our original CDC-AGO resulted in a 43 percent reduction in the abundance of parous and gravid *Ae. aegypti*. To improve the potential of the trap as a vector control and surveillance tool, we evaluated several modifications to the design in competitive assays performed under laboratory and semi-natural conditions. The following changes to the trap significantly increased capture efficiency: increasing the size of the trap entrance, altering the color of trap components, and increasing the volume/surface area of the aqueous bait. The use of olfactory baits other than hay infusion (eg. synthetic baits, other organic substrates) did not improve trap performance. In a field study, mean (± SE) numbers of adult *Ae. aegypti* females captured per trap per day were 1.16 ± 0.05 in the modified CDC-AGO (max. collection = 6.75), and 0.36 ± 0.02 in our original CDC-AGO (max. collection = 2.67). Semiweekly collections of *Ae. aegypti* females in the modified trap were more significantly correlated with cumulative rainfall 12 - 25 days prior to sampling than in the original CDC-AGO or collections of eggs in standard ovijars. In a second field test, average semiweekly capture rates were as high as 3.3 *Ae. aegypti* females per trap per day in the modified CDC-AGO. The modified CDC-AGO was highly attractive to gravid *Ae. aegypti* females for up to 18 weeks without need for maintenance.

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THE IMPACT OF ANTI-VECTOR INTERVENTIONS ON THE EFFECTIVE POPULATION SIZE OF MALARIA MOSQUITOES

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Malaria vector control in the form of indoor residual spraying (IRS) and insecticide treated net (ITN) distribution has the ability to significantly impact malaria transmission. Although a reduction in malaria mosquito abundance has been reported following vector control, we know little about the extent to which IRS and ITN distribution is capable of reducing the effective size (N_e) of mosquito populations. A nationwide malaria control program has been implemented in Equatorial Guinea under the Bioko Island Malaria Control Project and the Equatorial Guinea Malaria Control Initiative. Anti-vector interventions under these programs consist of IRS and ITN distribution. We examined the impact of these interventions on the effective size of three species of malaria mosquitoes in several locations on Bioko Island and the Equatorial Guinea mainland. Microsatellite data were collected for several time points from two populations on Bioko Island, and 5 populations on the mainland. These populations include four *Anopheles gambiae* populations, two *An. melas* populations and a single *An. moucheti* population. These data were analyzed using a coalescent-based Approximate Bayesian Computation which allows a comparison of various demographic models. A demographic model describing a recent reduction in effective population size provided the best fit for the populations analyzed. Both IRS and ITN distribution had a substantial impact on the effective size of *An. gambiae* populations, reducing effective population size over 5- fold in the populations examined. Additionally, the timing of this N_e reduction matches well the implementation of vector control. These results show that IRS and ITN have a remarkably large impact on the size of *Anopheles gambiae* populations and that much of their effectiveness can be ascribed to decreases in mosquito populations, rather than a reduction in contact between mosquitoes and their human hosts.

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EFFECTIVENESS OF VARIOUS CHEMICAL COMPONENTS AS ATTRACTANTS TO PHLEBOTOMINE SAND FLIES IN TWO DIFFERENT ECOLOGICAL AREAS OF PERU

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Sand flies are small bloodsucking flies in the family Phlebotominae (Diptera: Psychodidae) that transmit the pathogens of human diseases; e.g.,leishmaniasis, and bartonellosis. In the Peruvian Andes, cutaneous leishmaniasis and bartonellosis are endemic diseases and in some areas (Madre de Dios) of the Amazon Jungle, leishmaniasis is an endemic disease. Surveillance of the local sand fly fauna is essential to establish and maintain effective sand fly control programs that will then decrease human disease incidence. Sand flies are most effectively captured using human-landing methods, but there are many ethical concerns regarding this method. Alternatives to human-landing methods are: use of CDC light traps, and mouth aspiration of sand flies from resting sites in animal refuges or Shannon traps. However, these methods are time- and labor-intensive, and yield small quantities. In addition, capture of sand flies using the CDC light trap requires $C0_2$ (generated by dry ice or liquid nitrogen), which can be very difficult and expensive to obtain and transport to remote locations. For these reasons, it is imperative to find more efficient attractants that will attract *Lutzomyia* in quantities that will permit evaluation of population differences in abundance and diversity in time and space. The objective of this study was to compare, in two different ecological areas, the efficacy of CDC light traps using different combinations of lactic acid, octenol, benzaldehyde, and 4-methyl-2-pentanone with and without a $C0_2$ generator (yeast+sugar+water). We collected 2,755 sand flies in the Amazon Jungle area and 3,050 in the Andean area; we evaluated the difference between each treatment to determine which combinations enhanced the sand Oy capture-rates in each trap. According to these results, there is no statistical difference between treatments.

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BEHAVIORAL RESPONSES OF *AEDES AEGYPTI* USING EXPERIMENTAL HUTS IN AN URBAN ROWHOUSE DESIGN IN IQUITOS, PERU

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Dengue, transmitted by *Aedes aegypti,* is still one of the most impo11ant viral diseases worldwide. Due to the lack of an effective vaccine and treatment, mosquito control plays a vital role in the prevention of the disease. Today dengue control is based on reduction of adult vector populations using chemicals at toxic doses; however, insecticide resistance, environmental concerns. and adverse health effects are threatening the efficacy of this approach. Behavioral effects of insecticides at sub-lethal doses are being discussed and considered as one possible alternative. These effects include spatial repellency where adult mosquitoes are discouraged from entering a treated space thereby reducing human-vector contaand the probability of pathogen transmission.This study, conducted as part of a larger research program to field-validate a Push-Pull strategy to reduce *Ae. aegypti* inside homes, quantified spatial repellency effects under field conditions using a mark-release-recapture experimental hut design. The uniqueness of our approach is that the five experimental huts share an adjoining wall with another hut and have open eave gaps thereby creating a continuum of indoor space. Such a "rowhouse" configuration is typical of Iquitos and other urban environments where dengue is endemic. This complex environment allows us to measure intensity of distance effects of a spatial repellent tool when varied numbers of huts arc treated with the intervention. We report on the reduction of indoor *Ae. aegypti* densities within each of the individual hut "cells" using several spatial repellent chemicals and doses. This information will help to determine required coverage rates of a spatial repellent intervention and optimize the "push" component of the overall Push-Pull strategy in preparation for a pilot field trial in local homes.

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A COMMUNITY-WIDE REPELLENT TRIAL: EVALUATING THE EFFICACY AND USER-ACCEPTANCE OF LOW-COST MOSQUITO REPELLENT IN GHANA

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Commercial repellents have been available in developed countries for decades, but their application to infectious disease problems in less developed countries has been frustrated by doubts about their efficacy, affordability, and user-acceptance in reducing vector-borne diseases. We evaluated the efficacy, user-acceptance and epidemiological efficacy of

NO MAS (NM), a water-based formulation whose active ingredients are PMD (para-menthane-diol) and lemongrass oil against local anopheline vectors of malaria in rural Ghana.The field test was carried out in Korania, a community within the Kassena Nankana District of Northern Ghana. A total of 64 man-nights captured 10% (576) *Anopheles* mosquitoes in the treatment arm (NM users) and 90% (5486) in the control arm (non-NM users). The biting pressure of *Anopheles* on an unprotected individual in the area was 86 bites/man/night compared to 9 bites/ man/night when the person uses NM repellent. The average percentage level of protection (efficacy) of NM repellent in the community was 89.6%. After 37,710 user- days, a NM repellent user-acceptance rate of 96.7% was estimated for the community. The probability that members of the community at risk of vector borne infections can escape infection by using the repellent based intervention was calculated as *Fe*=0.997549 translating to about 0.002451% of malaria infections. In the absence of the repellent, the probability of escaping malaria infection was *Fe*=0.981256 translating to about 0.018744% of malaria infections.With a protection level of 90%, the relatively high epidemiological efficacy and the low cost of the repellent, when distributed and used en masse as in combination with ITNs and IRS in the country, it can offer a powerful synergy that can lead to the drastic reduction of malaria and lymphatic filariasis transmission in many rural poor communities in West Africa including Ghana.

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EFFECTS OF LONG-LASTING INSECTICIDE TREATED NETS IN A DIVERSE VECTOR ENVIRONMENT

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In Papua New Guinea (PNG) members of the Punctulatus Group, including *Anopheles punctulatus, An. koliensis, An. farauti* s.s., *An. farauti* 4 and *An. hinesorum* (formerly *An. farauti* 2), exhibit heterogeneities in distribution, biting behavior and malaria infection levels. The PNG National Department of Health recently launched a nationwide long-lasting insecticide-treated net (LLIN) program. This study aimed to evaluate the impact of the campaign on anopheline species density, composition, feeding behavior and malaria infectivity. Sentinel sites were chosen from 6 PNG provinces representing coastal, riparian, inland and highland regions. Entomological surveys were conducted one year prior to, and two years post-LLIN distribution. Host-seeking anophelines were collected by the landing catch method from 6pm to 6am (N=46,000). Adults were identified to morphospecies and confirmed by PCR-RFLP of the internal transcribed spacer 2 rDNA. Malaria infectivity was determined by circumsporozoite ELISA for *Plasmodium falciparum*, *P. vivax* 210 and *P. vivax* 247. A reduction in man-biting rates and *Plasmodium* infectivity was observed for each species and from each location in the first year following LLIN distribution. Most vectors exhibited a shift to earlier peak biting times. In villages with multiple vector species, a significant change in species composition was observed, with the earlier biters, members of the Farauti complex, dominating the collections. By the second year after LLIN distribution, man-biting rates increased, in some cases rivalling the pre-distribution levels. This shift in biting times will result in greater exposure to the vectors despite bednet usage and the changes in species composition may alter malaria transmission dynamics. The implementation of singular control strategies in areas with diverse vector communities requires careful consideration.

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ASSESSMENT OF MONKEYPOX KNOWLEDGE IN HEALTHCARE WORKERS FOLLOWING TRAINING - DEMOCRATIC REPUBLIC OF CONGO, FEBRUARY 2011

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Estimating the incidence of monkeypox in the Democratic Republic of Congo (DRC) has been difficult due to inconsistent reporting and a paucity of laboratory confirmation for suspected infections. To improve monkeypox surveillance we trained 59 healthcare workers from Tshuapa District, Equateur Province in monkeypox surveillance methods, clinical case recognition, and specimen collection. In order to evaluate the effectiveness of the training program, a survey of monkeypox-specific knowledge was conducted before and after training. In general, pre-training monkeypox knowledge was high, with an average score of 26/37, which increased by 11% post training. With respect to monkeypox symptoms, the greatest improvement was seen in the participants' ability to identify the following symptoms as being associated with monkeypox: deep seated lesions (39% vs. 93%; $p<0.0001$) and lymphadenopathy (47% vs. 89%; $p<0.0001$). After training, more participants were able to differentiate between clinical photos of monkeypox and varicella cases (44%, 93%; $p<0.0001$) and to identify vesicular fluid and crusts as the preferred specimens for clinical diagnosis of monkeypox (7% vs. 61%; $p<0.0001$). However, there was also an increase in the number of people spuriously identifying water as a mechanism for monkeypox transmission (12% vs. 29%; $p<0.02$). The reason for this increase is unclear. Future educational materials will be refined to better address deficiencies identified through this evaluation. The impact of this training on surveillance efficacy remains to be determined; however, during the 2 months after the training diagnostic specimens were submitted for 55% of reported cases, as opposed to 1% throughout 2010.

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THE SURVEY OF DISTRIBUTION CHARACTERISTICS OF MOSQUITOES AND MOSQUITO-BORNE ARBOVIRUSES IN NORTHEAST AND SOME OTHER AREAS OF YUNNAN PROVINCE

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To investigate the distribution characteristics of mosquitoes and mosquito-borne arboviruses in northeast and some other areas of Yunnan province, and to provide scientific basis for prevention and control of the arbovirus disease. Mosquitoes were collected in 6 counties of northeast and some other areas of Yunnan province in 2009. After classification and determination, all mosquitoes were used to viruses isolation. Positive isolates were identified by SDS-PAGE and RT-PCR, then sequenced and

phylogenetic analyzed. 4 genus (*Culex, Anopheles, Armigeres, Aedes*), 24 species, 18,562 mosquitoes were collected. *Cx. tritaeniorhynchus*, *An. sinensis* were main species in total, and their constituent ratios were respectively 58.37% (10,834/18,562) and 28.45% (5281/18,562). 15 strains of viruses were isolated from mosquito pools. By RT-PCR and phylogenetic analysis, 2 strains isolated from *Cx. tritaeniorhynchus* were identified as Japanese encephalitis virus (JEV, Genotypel). 1 strain isolated from *An. sinensis* was identified as Bannna virus. 12 strains were identified as *Cx. pipens pallens* densovirus (CppDNV), 9 strains of them were isolated from *Cx. tritaeniorhynchus* and 3 strains of them were isolated from *Anopheles sinensis*. *Cx. tritaeniorhynchus* and *An. sinensis* were the predominant species in the investigated areas. Japanese encephalitis virus, Bannna virus and CppDNV were isolated here. It was the first time that Japanese encephalitis virus had been isolated in northeast of Yunnan, China.

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CRIMEAN CONGO HEMORRHAGIC FEVER SURVEILLANCE IN KAZAKHSTAN, 2009-2010

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Crimean Congo Hemorrhagic Fever (CCHF) virus is a tick-borne pathogen that causes hemorrhagic fever symptoms with high fatality in hospitalized patients. While tick bites are an important means of transmission, little population-based data has been collected concerning tick bites and CCHF incidence. CCHF is endemic in Kazakhstan, with most cases occurring in the Southern Kazakhstan Oblast (SKO) region. Surveillance activities in this region included reporting of suspect and confirmed cases, and a registry of reported tick bites. We analyzed surveillance data for CCHF in Southern Kazakhstan, in order to better understand disease dynamics and evaluate tick bite reports as an indicator of CCHF risk. Line lists of CCHF case-patients were reviewed. Weekly summaries of tick bites reported in SKO during spring and summer of 2009-2010 were obtained, and the spatial and temporal incidence of tick bites and CCHF were compared. Twenty-two CCHF cases were reported in 2009 and 17 in 2010. Of the reported CCHF patients, 38% reported livestock exposures, 33% reported known tick exposures, 15% had nosocomial exposures and 14% had no risk factors identified. Weekly total reported tick bites in 2009 and 2010 correlated significantly with weekly CCHF occurrence (2009 $r = 0.58$, $p = 0.002$; 2010 $r = 0.60$, $p < 0.001$). Additionally, the incidence of tick bites was significantly higher in municipalities reporting CCHF cases than in those with no CCHF cases ($p = 0.01$). In conclusion, an analysis of CCHF surveillance data in Kazakhstan found a high number of reported tick bites, with spatial and temporal association between tick bites and CCHF cases. Public health measures should center on prevention of tick bites in people, increasing awareness of CCHF signs and symptoms in populations at risk of tick and livestock exposure, and adoption of infection control practices in the hospital setting.

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NOROVIRUS GASTROENTERITIS IN ECUADOR: DATA FROM A PILOT STUDY IN A RURAL DISTRICT

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Norovirus is the leading cause of both outbreaks and sporadic acute gastroenteritis in developed-countries. There are limited data on the epidemiology and burden of norovirus gastroenteritis in Latin America. We analyzed fecal samples for the presence of norovirus from children presenting with diarrhea in a rural District of Esmeraldas Province, Ecuador. The samples were from children aged 6-13 months in a surveillance cohort of 195 children nested within the ECUAVIDA birth cohort. A total of 190 stool samples from infants with diarrhea were analyzed by real-time reverse transcription-PCR to identify norovirus genogroup (G) I and II. Overall 43 (22.6%) samples tested positive for norovirus with 16 (8.4%) for norovirus GI and 27 (14.2%) for norovirus GII. The data suggest that norovirus infection is a significant cause of gastroenteritis in very young children in rural Ecuador.

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THE GERMAN ARBOVIRUS SURVEILLANCE AND MOSQUITO MONITORING PROGRAM, 2009 - 2010

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The aim of the program is to provide an early warning of the presence of arboviruses in Germany. The program compiles and analyses mosquito and arbovirus data collected over a number of successive years. This will provide a solid base to determine the underlying causes of the seasonal fluctuations in arbovirus activity and the relative abundance of the mosquito vector species. This information can then be used as a basis for vector control programs. During 2009 and 2010 we monitored mosquito vector populations and undertook surveillance of arbovirus activity mostly in South West Germany. Approximately 90,000 mosquitoes were captured and assayed for the presence of arboviruses. In 2009, Sindbis virus (SINV) and Batai virus (BATV) were isolated from *Culex* spp. and *Anopheles maculipennis s.l.*, respectively. The highest SINV infection rate (4.9) in the *Culex* mosquitoes was in the beginning of July. Phylogenetic analysis of the German SINV strains linked them with Swedish SINV strains, the causative agent of Ockelbo disease in humans. Analysis of partial S, M, and L segments of the German BATV strain showed that the sequences from all three segments were most closely related to BATV, indicating that the virus has not undergone reassortment. In contrast, only Usutu virus (USUV) was isolated in 2010 from *Culex* spp. and demonstrated to be related to USUV strains circulating in Austria and Italy. Further studies have to be conducted to estimate the veterinary and medical importance of SINV, BATV and USUV in the affected areas.

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IMMUNE FUNCTION AND MICRONUTRIENT STATUS OF PREGNANT WOMEN INFECTED BY HEPATITIS E VIRUS IN BANGLADESH BETWEEN 2001 AND 2010

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Hepatitis E virus (HEV) is the leading cause of acute viral hepatitis globally and results in severe morbidity and mortality in pregnant women. There is a paucity of longitudinal data examining the incidence and disease rate of HEV in cohorts of pregnancy in endemic areas. We studied serial sera collected within two prospective cohorts totaling 110,473 incident pregnancies enrolled large randomized trials in rural northwestern Bangladesh, between 2001 - 2007 (cohort A) and 2007 - 2010 (cohort B). An NIH research immunoassay was used to identify anti-HEV IgG status in early pregnancy, late pregnancy and 3 month postpartum venous blood specimens, drawn on a subsample of the larger cohorts. Of the 1,127 specimens available for testing in cohort A, 72 were anti-HEV seropositive at baseline, indicating a seroprevalence of ~6.4%. During

this period, 63 women were identified as potential seroconverters, suggesting an incidence rate of ~56 infections per 1000 person-years. In the more recent cohort B, 1100 were available for testing, revealing a ~6.1% seroprevalence in anti-HEV IgG at early pregnancy. Within this cohort, 40 women were identified as putative seroconverters, an incidence rate of 46 infections per 1000 person-years. Between the 2001 to 2006 cohort and the 2008 to 2010 cohort, the incidence of intrapartum HEV infections seems to be declining in rural Bangladesh, possibly reflective of improved sanitation. Cytokine and micronutrient analysis of the 2008 to 2010 cohort is ongoing to characterize the immunopathology of HEV infection. In the cohort A, 4 pregnant seroconverters with high antibody titers were evaluated for cytokine profiles, revealing elevated levels of pro-inflammatory cytokines compared to unifected controls and women who were seropositive at baseline. Treg-associated IL-10 levels also seem to be elevated in HEV-infected cases. Although no pregnancy-related mortality was observed in this nested cohort, analysis is ongoing to assess whether any sign of immune dysregulation or immune response inconsistent with late pregnancy is evident. Initial data suggests seroconverters seem to have lower baseline serum Zinc levels than their non-infected counterparts. Vitamin D and Copper (Cu) levels were also lower, although not statistically different. This data also seeks to elucidate population-based rates of HEV disease:infection ratios within a non-epidemic context, where this pathogen is ubiquitous.

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DIFFERENTIAL REPLICATION OF EPIZOOTIC VERSUS ENZOOTIC SUBTYPE IE VENEZUELAN EQUINE ENCEPHALITIS VIRUSES IN EQUIDS

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The etiologic agents during major Venezuelan equine encephalitis virus (VEEV) outbreaks are associated with subtypes IAB and IC, while subtype IE strains are generally considered enzootic, equine-avirulent, and incapable of exploiting horses as amplification hosts. However, Mexican epizootics on the Pacific Coast were shown to originate from equine-virulent subtype IE VEEVs. To determine whether the virulence of these subtype IE VEEVs correlated with the development of viremia, equine pathogenesis studies were performed, in which groups of horses were inoculated subcutaneously with one of three VEEV strains: 1) MX01-32, an unpassaged subtype IE strain genetically and geographically related to the Mexican outbreaks, 2) 68U201, a related enzootic subtype IE VEEV from Guatemala, and 3) 3908, an epizootic subtype IC VEEV strain from the last major equine-amplified epidemic. MX01-32-infected horses developed a febrile response and viremia that was comparable in titer to and earlier than that induced by 3908. To determine whether these *in vivo* findings could be correlated to the replication kinetics of these viruses *in vitro*, equine peripheral blood monocyte cultures were infected with either: 1) vesicular stomatitis virus (VSV) [a positive control virus known to replicate efficiently in equine monocytes], 2) 3908, 3) ZPC738 (an enzootic subtype ID VEEV), 4) 68U201, or 5) MX01-32. Culture supernatants were collected and titered by plaque assay. The Mexican strain of subtype IE VEEV (MX01-32) replicated to a higher titer than the enzootic Guatemalan strain (68U201), corroborating evidence that the virulence of epizootic subtype IE viruses depends on virus replication. To quantify the relative number of infected monocytes, indirect fluorescent antibody (IFA) assays were performed, which showed that, regardless of VEEV subtype, there were relatively few infected cells compared to the total number of cells in culture. These results suggest an intrinsic mechanism of modulating viral replication in equids, which may be a critical factor for the successful development of intervention strategies that protect human populations from future outbreaks.

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MULTIPLEX MICROSPHERE IMMUNOASSAYS FOR THE DETECTION OF IGM AND IGG TO ARBOVIRUSES

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A variety of techniques have been developed over the past 40 years for the serodiagnosis of arboviruses. These include immunofluorescence assay, complement fixation test, hemagglutination inhibition assay, plaque reduction neutralization test, and IgM and IgG enzyme-linked immunosorbent assays (ELISAs). The most recent addition to the menu of tests is the microsphere assay (MIA) which uses the Luminex platform. The use of combined serologic testing data is currently the method of choice for laboratory diagnosis of arboviruses. MIAs have been used as screening tools for arboviruses over the past 5 years. A number of US State and government labs including the CDC have used a duplex IgM test for West Nile (WN) and St. Louis encephalitis (SLE) viruses, and have participated in proficiency testing using this method. The speed and ease of use of this platform have made these tests attractive for expansion to other arboviruses, where viral antigens of interest can be incorporated into the testing battery as needed. The creation of IgM and IgG multiplex MIAs allow for a comprehensive array of arboviral infections to be tested for concurrently. Here we report the development of multiplex assays for IgM and IgG to 6 flaviviruses, 6 alphaviruses, and 1 bunyavirus of human importance, incorporating validation results for its practical use in geographic batteries. Internal test controls were included in the assays to boost confidence in the results. Samples from previous diagnostic submissions were used to generate MIA data, which were compared to those of IgM and IgG ELISAs and to the overall laboratory diagnoses for the patients. Six classification methods were compared to determine which performed best for this application, with linear kernel support vector machines proving to be the best for the geographic batteries. The analyses will be presented.

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ORTHOBUNYAVIRUSES ARE A COMMON CAUSE OF INFECTION IN DOMESTIC ANIMALS IN THE YUCATAN PENINSULA OF MEXICO

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A serological investigation was performed to determine the seroprevalence of various orthobunyaviruses in domestic animals in the Yucatan Peninsula of Mexico. The study was performed using an archived collection of sera taken from 256 domestic animals (182 horses, 31 sheep, 1 dog, 37 chickens and 5 turkeys) at multiple study sites in the Yucatan Peninsula between September 2007 and October 2008. All sera were initially screened at a single dilution (1:20) by plaque reduction neutralization test (PRNT) using five orthobunyaviruses: Cache Valley virus (CVV), Maguari virus (MAGV), South River virus (SORV), Kairi virus (KRIV) and Wyeomyia virus (WYOV). If neutralizing antibodies were detected, the sample was further diluted and subsequent PRNTs were performed to determine the end-point $PRNT_{90}$ titer. Remarkably, antibodies to orthobunyaviruses were detected in most of the sera tested. Of the 182 horses analyzed, 83 (46%) were seropositive for CVV, 18 (10%) were seropositive for MAGV, 2 (1%) were seropositive for SORV, 64 (35%) had antibodies to an undetermined orthobunyavirus and 15 (8%) were negative. Of the 31 sheep analyzed, 8 (26%) were seropositive for CVV, 4 (13%) were seropositive for SORV, 15 (48%) had antibodies to an undetermined orthobunyavirus and 4 (13%) were negative. The single dog analyzed in this study was seropositive for SORV. Additionally, 4 (11%) chickens had antibodies to an undetermined

orthobunyavirus and 1 (20%) turkey was seropositive for CVV. These data indicate that orthobunyaviruses are a common cause of infection in some species of domestic animals in the Yucatan Peninsula of Mexico.

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ALPHAVIRUS INFECTION AMONG PEDIATRIC ENDEMIC BURKITT'S LYMPHOMA IN KENYA

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Arboviral infections have been implicated as precursors to the onset of lymphoma. In regions of equatorial Africa, endemic Burkitt's lymphoma (eBL) and arboviral infection overlap in both geography and demography. The most prevalent childhood cancer in Kenya is eBL. Alphaviruses, such as chikungunya virus (CHIKV), are also common in Kenya. During a previous arboviral serosurvey conducted in western Kenya (N=122, median age=8.59, mean age=9.04), 20% of children were positive for CHIKV-specific IgG by immunofluorescence (IFA) testing (N=24, median age=9.62, mean age=9.55). A sample of pediatric eBL-positive Kenyan children was tested for CHIKV IgG by IFA (N=48, median age=6, mean age=6.62). eBL samples were more likely to be CHIKV IgG positive (35% vs. 20%, p =0.0453). Of the CHIKV positive eBL samples, 59% were boys and 41% were girls. Gender was not associated with CHIKV-status (p=0.7611), nor was prior malaria treatment (p=0.1765). Curiously, eBL children who were CHIKV seropositive had a higher survival rate (76% vs. 42%, p=0.0339). After controlling for tumor site, CHIKV was still associated with eBL survival. Given these preliminary data, chikungunya virus exposure is associated with endemic Burkitt's lymphoma among children in this study area. Further investigation of the effects of alphaviruses and other arboviruses on eBL incidence, prevalence and outcomes may be warranted given the high prevalence of both arboviruses and eBL in the region.

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VIRAL HEMORRHAGIC FEVER SURVEILLANCE IN UGANDA (2010-2011)

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Uganda is endemic for viral hemorrhagic fevers (VHFs), including Ebola, Marburg, Rift Valley Fever (RVF) and Crimean Congo Hemorrhagic Fever (CCHF) viruses. In order to enhance the ability to identify and rapidly test for VHF's, in July 2010 the Viral Special Pathogens Branch (VSPB), CDC, the Uganda Virus Research Institute (UVRI), and the Ministry of Health (MOH) established a National VHF surveillance program. Since July 2010, VSPB Uganda has established 6 sentinel VHF surveillance sites and trained 28 clinical and laboratory staff on the identification, reporting, infection control, and clinical sample collection procedures for suspect VHF cases. The laboratory has also processed more than 70 clinical samples for suspect VHF. Notably, the program was alerted in early October, 2010 of an "unknown illness" in Northern Uganda and performed rule-out testing on 55 suspect cases. Acute diagnostic testing for Ebola hemorrhagic fever was performed by PCR and antigen detection and for Marburg hemorrhagic fever by PCR on all clinical samples, indicating that neither Ebola nor Marburg virus was the etiologic agent. Among acute samples with an known onset date, the median time from symptom onset to sample collection was 3 days, and median time for all acute samples tested, from either collection or case report to diagnostic rule-out for EHF and MHF was 6 days (by PCR), 13 days (by serology). A subset of 17 samples was subsequently sent to CDC, Atlanta for further testing for viral hemorrhagic fever; all were demonstrated negative for Rift Valley Fever (by PCR and IgM ELISA) and Crimean Congo hemorrhagic fever (by PCR). Next generation sequencing (NGS) was employed to detect pathogen genomic material amplified from patient serum for Yellow fever virus (YFV) in one of four patient sera tested; the first YFV case detected in Uganda since 1964. The Uganda VHF surveillance program has the ability to rule-out commonly suspected viral hemorrhagic fevers in a timely manner and continues to receive suspect VHF cases reports and perform diagnostic testing to rule out VHFs.

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TRANSFORMING GROWTH FACTOR-β AND INTERLEUKIN-10 ALTER HANTAVIRUS CARDIOPULMONARY SYNDROME DISEASE SEVERITY

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Sin Nombre virus (SNV) was first identified in 1993 in the Four Corners region of North America as an etiologic agent of hantavirus cardiopulmonary syndrome (HCPS). Infection is associated with high levels of inflammatory cytokine staining in human pulmonary autopsy specimens, suggesting HCPS is an immunopathology. The reservoir of SNV is the deer mouse (Peromyscus maniculatus), which develops persistent infection without pathology. Experimental data showed increased expression of transforming growth factor beta-1 (TGFβ1) in virus-specific helper T cells from these animals. The Syrian golden hamster (Mesocricetus auratus) has been used as an HCPS model with Maporal virus (MAPV). Our hypothesis predicted use of TGFβ1 or interleukin-10 (IL-10) as a therapeutic agent would attenuate disease severity. Gene expression in both lung and spleen suggested an innate immune response. Spleens had increased chemokine expression of IL-12 (both p35 and p40 subunits), as well as p27 and Eif2ak2 that indicated an attempt by cells to limit viral protein synthesis and cell division. Lungs had increased expression of CXCL10, ICAM-1, PECAM and VEGF which also suggest attraction of leukocytes. By day 10 there was an increase in adaptive immune cytokines in both spleens and lungs. Spleens had increased expression of IL-6 and IL-21 genes, suggestive of a CD4+ T cell response. Lungs had a notable increase in MHC-II gene expression. The administration of TGFβ1 appeared to suppress expression of IL-12 in spleens and MHC-II in lungs. Hamsters infected with MAPV and treated with TGFβ1 had decreased lung congestion and pleural fluid, although no significant attenuation of disease was observed. Administration of IL-10 resulted in increased lesion score and no suppression of gene expression.

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MYELOID CELL RELA PROMOTES ROSS RIVER VIRUS-INDUCED MUSCULOSKELETAL DISEASE

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Ross River virus (RRV) and chikungunya virus (CHIKV) are mosquito-transmitted alphaviruses that cause debilitating rheumatic disease in humans. Studies in humans and animal models suggest that macrophages have critical pathogenic and protective roles in alphavirus-induced rheumatic disease, but specific macrophage effector mechanisms that mediate these effects have not been defined. Nuclear factor-κB (NF-κB) is a transcription factor that regulates the activation of myeloid cells during inflammatory reactions, and inhibitors of NF-κB have been shown to reduce the severity of RRV-induced disease. To investigate the role of myeloid cell NF-κB activity in the pathogenesis of RRV/CHIKV infection, we bred mice to delete the canonical NF-κB subunit RelA (p65) specifically from myeloid cells (LysMCre;RelAflox). Following

RRV infection, LysMCre;RelAflox mice had less severe altered gait and deficits in gripping ability, as well as improved weight gain compared to control mice. These findings suggest that RelA activity in myeloid cells promotes RRV-induced rheumatic disease. Interestingly, despite less severe rheumatic disease signs, LysMCre;RelAflox mice had higher viral loads in tissues. Taken together, our data suggest that RelA regulates myeloid cell effector functions that mediate tissue injury and/or virus control during RRV infection. We propose that further studies in this model may allow us to define specific effector mechanisms by which myeloid cells mediate protection or pathology following RRV/CHIKV infection, leading to a better understanding of the pathogenesis of these infections and aiding in the rational design of targeted therapeutics.

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LIVE BIRD MARKET ENVIRONMENTAL SAMPLING: A TOOL FOR POULTRY INFLUENZA SURVEILLANCE

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Since 2007, Bangladesh has annually reported influenza A/H5 poultry outbreaks during January-April with sporadic infections among humans. We conducted live bird market surveillance to identify circulating influenza viruses in poultry. From May 2009 to March 2011 we collected environmental samples every month from three rural and eight urban live bird markets in Bangladesh. The weekly rural markets were distributed across the country and were selected based on their higher poultry population density. Eight urban markets were in Dhaka city, where the dealers daily brought poultry from different districts. Once a month, we swabbed poultry cages, feed and water trays, and fecal material from the poultry stalls of the birds and collected 10 swabs as convenience samples of multiple sites and pooled them into one environmental sample per market. We conducted real-time reverse transcription polymerase chain reaction (rRT-PCR) for identifying influenza A viruses and H5 sub-type. We collected 213 pooled environmental samples from the live bird markets. Of the 72 environmental specimens collected from rural markets, 32 (44%) were rRT-PCR positive for influenza A viruses and of these, two (3%) were positive for H5 virus. Of the 141 environmental specimens collected from eight urban live bird markets, 100 (71%) were rRT-PCR positive for influenza A viruses and of these, 49 (35%) were H5 virus positive. The environmental samples collected from the urban live bird markets were three times more likely to be influenza A positive compared with the rural samples (p=<0.001). The majority (n=47, 96%) of the influenza A/H5 virus positives in Dhaka markets were identified during October and March, the cooler months (mean temperature: 23 degree C; range: 20-27 degree C) of the year in Bangladesh. During the same time period, 171 (89%) poultry outbreaks were reported nationally. In conclusion, we frequently identified influenza A/H5 in the urban live bird markets concurrent with seasonal outbreaks reported throughout Bangladesh. Urban live bird markets serve as collection points of poultry from throughout the country may act as sentinels for circulating influenza viruses. Live-bird markets may be high-risk sites for harboring influenza viruses and prime sites for interventions aimed at preventing transmission in poultry.

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HEPATITIS E VIRUS DETECTION AND CHARACTERIZATION IN SEWAGE FROM VELLORE, SOUTH INDIA

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Hepatitis E virus (HEV) is enterically transmitted and causes an acute, self-limiting hepatitis. In developing countries, HEV is endemic. The virus is hyperendemic in India leading to frequent water-borne epidemics and high rates of sporadic acute hepatitis. Fecal shedding of HEV from both humans and animals maintains the virus in sewage. Since sewage systems are important points to monitor enteric pathogens transmitted through water, we carried out a monthly sampling and testing for HEV in sewage. For this study to detect and characterize HEV in sewage and compare its frequency and seasonal pattern with another enteric pathogen, rotavirus and HEV were investigated in sewage from Vellore, a city in the state of Tamil Nadu in South India. From November 2009 to October 2010, 12 sewage samples were collected each month from the major sewage outlets, where the city's untreated sewage is discharged. A total of 144 raw sewage were tested for HEV RNA and rotavirus. Viral particles in Sewage were pelleted using ultra-centrifugation based concentration method. The total RNA extracted was subjected to polymerase chain reaction using specific primers for HEV and rotavirus. HEV strains isolated from sewage were sequenced. The overall prevalence of HEV RNA in sewage was 55.5% and that of rotavirus was 77%. HEV RNA was identified more often during the summer (81.2%) compared to the monsoon (14.5%) (P <0.001), while rotavirus was found more often in winter (97.9%) than during the monsoon (50%) (P <0.001). All the HEV strains isolated from sewage belonged to genotype 1. They were genetically closely related to HEV strains from Swedish nationals who were infected while travelling in India and HEV strains implicated in a large outbreak in Nellore, South India. The frequency of HEV in sewage from Vellore, South India was higher than reports from other parts of India. HEV strains in sewage from Vellore are of human, not animal, origin. This study underscores the need for preventive measures to protect drinking water from sewage contamination, particularly in the summer.

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RTS,S/AS01 MALARIA VACCINE CANDIDATE PHASE III EVALUATION: EFFICACY AGAINST CLINICAL MALARIA IN AFRICAN CHILDREN 5-17 MONTHS OF AGE

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In phase II clinical trials, the RTS,S/AS01 malaria vaccine candidate provided protection against malaria in African children living in malaria endemic regions. Recent results showed 39% and 42% vaccine efficacy (VE) against first episode and all episodes clinical malaria respectively, over 12 months. Investigators are now conducting a large multi-center phase III randomized, double-blind, trial. The trial has enrolled 15,460 children in two age categories, 5-17 months, and 6-12 weeks. We will present results from the primary analysis on 6000 children aged 5-17 months, during 12 months following vaccination. We will focus on VE measures using primary and secondary case definitions of clinical malaria. This ongoing phase III randomized, double-blind, controlled trial is being conducted at 11 sites in 7 African countries, representing diverse malaria transmission settings. Children aged 5-17 months whose parents provided informed consent were randomized 2:1 to receive the RTS,S/AS01 candidate malaria vaccine or comparator (rabies) vaccine, administered monthly for 3 doses. Clinical malaria episodes were captured by passive case detection. The primary case definition was *P. falciparum* parasitemia >5000 parasites/μL in an unwell child brought to a study clinic with temperature ≥37.5°C, or a case meeting a standardized primary case definition of severe malaria disease. Secondary case definitions differ in parasite density thresholds: >0, >500, >20,000 parasites/μL.VE will be assessed using Cox regression models (first episodes) and negative-binomial regression (multiple episodes). Anti-CS antibody titers were measured with a validated ELISA test at enrollment and one month post vaccine dose-3. VE against the first or only episode of clinical malaria and against multiple episodes of clinical malaria will be presented for all parasite density thresholds. Primary analysis will be according to protocol (12 months post-dose 3). An intention to treat analysis (14 months post-dose 1) will also be presented. Anti-CS antibody response at 1 month post dose-3 will be presented.

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RTS,S/AS01 MALARIA VACCINE CANDIDATE PHASE III EVALUATION: EFFICACY AGAINST SEVERE MALARIA DISEASE

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The RTS,S/AS01 candidate vaccine is being developed with the aim of reducing the burden of malaria including the approximately 800,000 deaths that occur each year, mainly in African infants and young children. Vaccine efficacy against the most severe forms of disease will be an important driver of the vaccine implementation decision process. The protective efficacy of RTS,S/AS01 against severe malaria is currently being evaluated in a multicenter Phase 3, randomized, controlled, double blind trial in children aged 5 to 17 months or 6 to 12 weeks old at first vaccination, across 11 research sites in 7 African countries. A standardized clinical algorithm for evaluation of sick children is being used to capture severe malaria cases in children presenting to clinical facilities. The primary endpoint definition of severe malaria includes a positive *P. falciparum* parasitemia over 5000/µL, specific clinical and laboratory markers associated with a risk of an adverse outcome and the absence of important co-morbidities. Vaccine efficacy against severe malaria will also be evaluated using additional secondary case definitions which include other parasitemia thresholds or allowing for inclusion of cases presenting with comorbidities. Here, we present an initial analysis of vaccine efficacy against severe malaria which will be based on an analysis of the entire trial population (ATP cohort for efficacy) up to the point when approximately 250 cases have been accumulated, as well as upon 1 year follow up after the primary vaccination schedule in children in the 5-17 months age group. Findings from the Phase III RTS,S/AS01 trial will provide key evidence on whether the vaccine can play a role in reducing the risk of severe malaria and associated co-morbidities in the target population.

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STRAIN-SPECIFIC *PLASMODIUM FALCIPARUM* GROWTH INHIBITION AMONG MALIAN CHILDREN IMMUNIZED WITH THE FMP2.1/AS02A VACCINE

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Additional authors: Issa Diarra, Amadou Niangaly, Amagana Dolo, Modibo Daou, Youssouf Tolo, Mady Sissoko, Shannon Takala-Harrison, Olivier Godeaux, Brent House, Jason W. Bennett. The blood stage malaria vaccine FMP2.1/AS02A, comprised of recombinant *Plasmodium falciparum* apical membrane antigen 1 (AMA1) and the adjuvant system AS02A, had strain-specific efficacy against clinical malaria caused by *P. falciparum* with AMA1 sequence corresponding to the vaccine strain 3D7. To evaluate a potential correlate of protection, we measured the ability of sera to inhibit the growth of 3D7 and FVO strains *in vitro* using high-throughput growth inhibition assay (GIA) testing. Sera from both AMA1 vaccine and control rabies vaccine groups were analyzed at baseline and eight subsequent time points over two malaria seasons. Baseline GIA against the vaccine strain 3D7 was similar in both groups, but a significantly higher proportion of AMA1 vaccinees had 3D7 GIA activity above a pre-determined threshold of 15% thirty days after the last vaccination (day 90) compared to the control group (49% vs 16%). From baseline to day 90 (corresponding to the start of the malaria season), 3D7 GIA in the AMA1 group increased 3.6-fold compared to 1.3-fold in the control group ($p<0.0001$). This increase was not associated with efficacy against all clinical malaria, but was associated with efficacy against clinical malaria with 3D7-type AMA1 sequence with respect to eight immunologically important amino acid residues in all participants ($p=0.01$). Baseline GIA against the FVO strain was also similar in both groups, but did not increase in either group. Analyses of GIA at additional time points are underway and may further elucidate the association of 3D7 GIA with protection. These results provide a potential immune correlate of strain-specific protection against clinical malaria for a blood stage vaccine, and will inform the development of more broadly protective next-generation malaria vaccines.

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PHASE 1/2A OPEN-LABEL DOSE SAFETY, REACTOGENICITY, IMMUNOGENICITY AND EFFICACY OF THE CANDIDATE *PLASMODIUM VIVAX* MALARIA PROTEIN 001 (VMP001) ADMINISTERED INTRAMUSCULARLY WITH GSK BIOLOGICALS' ADJUVANT SYSTEM AS01$_B$ IN HEALTHY MALARIA-NAÏVE ADULTS

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A vaccine to prevent infection and disease caused by *Plasmodium vivax* is needed both to reduce the morbidity caused by this parasite and as a key component in efforts to eradicate malaria worldwide. *Vivax* malaria protein 1 (VMP001) is a novel chimeric protein that incorporates the N- and C-terminal parts of the circumsporozoite (CS) protein and a truncated repeat region that contains repeat sequences from both the VK210 (type 1) and the VK247 (type 2) parasites. Following promising preclinical findings, we conducted a first-in-human Phase 1/2a vaccine study of VMP001 formulated in the GSK Adjuvant System AS01$_B$. The study was designed to incorporate dose-escalation, evaluating 3 antigen doses. A total of 30 volunteers were divided into 3 groups (10 in each group) and given 3 intramuscular injections at defined intervals of 15 µg, 30 µg, and 60 µg respectively, all in 500 µL of AS01$_B$ at each immunization. A *P. vivax* infected mosquito challenge was performed in 6 infectivity control volunteers and all volunteers from the 3 vaccine groups 14 days following the third immunization. The vaccine was shown to be safe and immunogenic; although it did not induce sterile protection, a small but consistent increase in pre-patent period was observed in some subjects. Volunteers who developed parasitemia were treated with chloroquine and primaquine as soon as parasites were identified on screening blood smears. This trial was the first testing of a *P. vivax* candidate vaccine in the clinic in conjunction with the *P. vivax* sporozoite challenge model. The ability to challenge vaccine recipients will accelerate the process of *P. vivax* vaccine development, allowing better selection of candidate vaccines for

advancement to field trials. The safety, reactogenicity, immunogenicity against VMP001, and efficacy of the vaccine against a *P. vivax* sporozoite challenge are reported.

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LACK OF PROTECTIVE EFFICACY OF AN ADENOVIRUS-VECTORED *PLASMODIUM FALCIPARUM* MALARIA VACCINE IN THE ABSENCE OF DNA PRIMING

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Malaria remains one of the world's major public health problems with a vaccine urgently needed. A multi-stage, multi-antigen prototype adenovirus (serotype 5)-vectored vaccine, designated NMRC-M3V-Ad-PfCA (AdCA), is under evaluation by the U.S. Military Malaria Vaccine Program (USMMVP). The vaccine is comprised of adenovectors encoding two malaria antigens: 1) circumsporozoite protein (CSP), expressed in sporozoite and early liver stages, and 2) apical membrane antigen 1 (AMA1), expressed in sporozoite, liver and erythrocytic stages. The USMMVP recently demonstrated that priming with a DNA vaccine encoding the same two malaria antigens followed by boosting with the AdCA vaccine sterilely protected 4 of 15 human volunteers against sporozoite challenge in association with strong CD8+ T cell-dependent interferon (IFN)-γ ELISpot responses. The current trial evaluated the protective efficacy of the AdCA vaccine given without DNA priming. A single dose of AdCA (1 x 10^{10} pu/antigen) was administered to 20 healthy, malaria naïve, Ad5 seronegative volunteers. Four weeks later 18 immunized and 6 unimmunized infectivity controls underwent homologous *Plasmodium falciparum* sporozoite challenge by the bites of mosquitoes. The AdCA vaccine was safe and well-tolerated. IFN- γ ELISpot responses were higher following AdCA in the absence of a DNA prime (CSP range 34-2508 sfc/10^6 PBMCs, geometric mean 236; AMA 1 range 399-4456 sfc/10^6 PBMCs, geometric mean 1102) than when the prime had been given in the previous trial (CSP range 5-375 sfc/10^6 PBMCs, geometric mean 43; AMA1 range 14-1165 sfc/10^6 PBMCs, geometric mean 177). However, all challenged volunteers became parasitemic with no significant delay to patency in the immunized compared with the control group. Although the AdCA vaccine administered alone stimulates quantitatively superior ELISpot responses against whole proteins than when following DNA priming, it does not confer sterile protection. This suggests a qualitative difference in the responses. The nature of the protective T cell response requires further investigation.

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LONGEVITY AND COMPOSITION OF CELLULAR IMMUNE RESPONSES FOLLOWING EXPERIMENTAL *PLASMODIUM FALCIPARUM* MALARIA INFECTION IN HUMANS

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Cellular responses to *Plasmodium falciparum* parasites, in particular interferon-gamma (IFNγ) production, play an important role in anti-malarial immunity. However, clinical immunity to malaria develops slowly amongst naturally exposed populations, dynamics of cellular responses in relation to exposure are difficult to study and data about the persistence of those responses are controversial. Here we assessed the longevity and composition of cellular immune responses following experimental malaria infection in human volunteers. We conducted longitudinal studies of cellular immunological responses to sporozoite (*Pf*Spz) and blood-stage (*Pf*RBC) malaria parasites in naïve human volunteers undergoing single or multiple experimental *P. falciparum* infections under highly controlled conditions. We show that induced cellular responses to both *Pf*Spz and *Pf*RBC remain present up to 14 months after even a single malaria episode. Remarkably, not only 'adaptive' but also 'innate' lymphocyte subsets contribute to the increased IFNγ response, including αβT cells, γδT cells and NK cells. The majority of responding T-lymphocytes express an effector memory phenotype both early and late post-infection and CD4+ cells outnumber CD8+ cells. We established that both γδT cells and αβT cells independently contribute to immunological memory. Finally, we demonstrate that malaria infection induces and maintains notable pluripotent (IFNγ+IL-2+) effector memory responses against both *Pf*RBC and *Pf*Spz, found previously to be associated with protection. These data demonstrate that cellular responses induced by infected mosquito bites can be readily induced and are long-lived with a continued interdependence between adaptive and (semi-)innate lymphocyte subsets.

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ADAPTIVE CLINICAL TRIALS OF THREE PFSPZ PRODUCTS FOR DEVELOPMENT OF A WHOLE SPOROZOITE VACCINE THAT PREVENTS *PLASMODIUM FALCIPARUM* INFECTION, DISEASE AND TRANSMISSION

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An ideal, single stage vaccine for elimination of *Plasmodium falciparum* (Pf) would prevent infection at the pre-erythrocytic stage of the life cycle, thereby preventing all Pf-caused disease and Pf transmission. The only approach to immunization that induces > 90% protection against infection that is sustained for at least 10-28 months, is immunization by mosquito bite with whole Pf sporozoites (SPZ) of two types. The first, radiation-attenuated PfSPZ, invades hepatoctyes and expresses new proteins, but cannot replicate. The second type fully develops in hepatocytes, producing tens of thousands of merozoites that invade erythrocytes, but cannot fully develop within erythrocytes, because the parasites are killed by chloroquine taken by during immunization. Sanaria was founded to develop PfSPZ vaccines. The 1st vaccine is based on radiation attenuated PfSPZ. The first task accomplished was production of PfSPZ that met regulatory and cost of goods requirements. PfSPZ Vaccine comprises radiation attenuated, aseptic, purified, cryopreserved PfSPZ. In the 1st clinical trial in 80 volunteers it was safe, and well tolerated. However, it was sub-optimally immunogenic and protective due to inefficient administration. A 2nd product, PfSPZ Challenge, comprises non-irradiated, fully infectious PfSPZ. PfSPZ Challenge infected volunteers after intradermal administration by needle and syringe. However, administration was not optimally efficient. A 3rd product, PfSPZ-CVac, comprises PfSPZ Challenge administered to volunteers while receiving chloroquine chemoprophylaxis. Assessment of these three products in interactive and adaptive clinical trials will facilitate progress toward optimizing administration and dosage regimen of all three whole PfSPZ products, as well as those developed in the future from genetically altered parasites, thereby speeding licensure of one or more PfSPZ-based vaccines. In 2011-

2012 we will execute clinical trials of all three PfSPZ products at multiple clinical trials centers in N. America, Europe, and Africa. Plans and progress will be described.

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EVALUATION OF CHOLERA SEVERITY IN A HIGHLY AFFECTED HAITIAN POPULATION

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Exposure to toxigenic *Vibrio cholerae* O1 results in a wide spectrum of illness, from asymptomatic infection to severe disease. The variant strain of *V. cholerae* causing the epidemic in Haiti may be more virulent than previous El Tor biotype strains, which were estimated to cause severe disease in 2% of those infected. Grand Saline, a commune in Artibonite Department reported a high cholera attack rate (19.0%) between Oct. 16, 2010 and Feb. 19, 2011. We conducted a cross-sectional survey in this commune (estimated population 21,131) from Mar. 22 to Apr. 6, 2011 to characterize disease severity. We interviewed 2,543 residents ≥2 years old in 1,228 households selected by multistage sampling from 13 villages and collected serum from 2,464 (97%). The median age of participants was 23.5 years (range 2-90); 59.0% were female. A healthcare provider diagnosis of cholera was reported by 466 (18%) of all respondents, including 187 (18%) males and 279 (19%) females, and 239 (16%) of those <age 30 compared with 227 (21%) of those ≥30 years old ($p<0.01$). Any antacid use was reported by 103 (23%) of those with cholera versus 292 (15%) of those without cholera ($p<0.01$). Among the 466 respondents with a cholera diagnosis, 429 (92%) reported rice-water stool (median maximum 24-hour stool frequency = 7), 361 (78%) painful leg cramps, and 225 (48%) vomiting more than once. For treatment, 405 (87%) reported using oral rehydration salts, 315 (68%) receiving antibiotics, 213 (46%) receiving IV fluids, and 191 (41%) overnight hospitalization; 157 (33%) reported both IV fluids and hospitalization. Persons with severe disease (IV fluids and hospitalization) represented 6% of the study population. From a subset of 589 households with information about all 2,396 household members, 311 people were diagnosed with cholera (13.0%) and 13 cholera deaths were reported (case fatality ratio, 4%). Severe disease was common, occurring in 6% of the study population rather than 2% of those infected, as seen with previous El Tor strains. Serologic measures of exposure to *V. cholerae* will be examined.

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REVIEW OF CHOLERA CASES AND DEVELOPMENT OF QUALITY ASSURANCE TOOL FOR CHOLERA CARE AT HÔPITAL ALBERT SCHWEITZER, DESCHAPELLES HAITI

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The first cholera outbreak in Haiti in decades was confirmed this past October. Hôpital Albert Schweitzer (HAS) received a high volume of patients in the initial months of the epidemic, and records of patients meeting the WHO definition of cholera from October 20, 2010 to January 12, 2011 were reviewed. Demographic data included: age, sex, residence, and travel time to HAS. Clinical data included: days ill before presentation, length of stay, treatment protocol level (based on severity of condition at presentation), liters of intravenous fluid (IVF) received, use of antibiotics, concurrent illness, and outcome. Length of stay, use of antibiotics, liters of IVF, and protocol number were used as surrogate markers for illness severity. A total of 514 charts were located and reviewed. Mean age was 37 years, and 54% were male. The rural sections of Belanger, Liancourt, Terre Nette, and Bastien had the highest percentage of cases represented, with travel time to HAS of 10 minutes to 8 hours. Mean duration of illness before presentation was 1 day (range 0-6), and average length of stay was 3 days (range 0-15). A total of 78% of patients received IVF, with an average amount of 8.5L (range 0-47L). About half (47%) of patients received antibiotics, and overall mortality was 0.6%. Having a concurrent illness was a statistically significant predictor of all four markers of illness severity. Age was found to be a predictor of protocol assignment and antibiotic prescription. Based on information gleaned from this review and the comments of clinicians providing care, a quality assurance and documentation tool was developed for patient care. This tool consisted of a simple, single-page form, which included both documentation of the patient's clinical criteria at admission and a guide to assignation of treatment level. It is hoped that this review will contribute to better understanding of the ongoing epidemic and that utilization of a standardized form will increase efficiency of both patient care and data collection at HAS.

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PROTECTION AGAINST EPIDEMIC CHOLERA IN POST-EARTHQUAKE PORT-AU-PRINCE, HAITI, 2010

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On January 12, 2010, a magnitude 7.0 earthquake struck metropolitan Port-au-Prince, Haiti, destroying vital water and sanitation infrastructure and leaving 1.3 million people displaced. On October 21, 2010 toxigenic *Vibrio cholerae* O1 was confirmed to be the cause of a large outbreak of acute watery diarrhea in Artibonite department. On November 7, 2010, cholera was first reported in Port-au-Prince and by December 15, nearly 20,000 cases were reported in the city. We conducted a case-control study to examine exposures associated with cholera in this crowded urban environment. Between December 15-19, 2010, we enrolled cases who were persons ≥5 years old with acute, watery diarrhea admitted to the GHESKIO Cholera Treatment Center in the slum of Cité de Dieu and two age-, sex- and neighborhood-matched controls per case. We used a standard questionnaire to gather information about food and beverage exposures in the 3 days before illness onset, and water, sanitation and hygiene practices. We enrolled 53 cases and 106 controls. The median age of cases was 29 years (range 6-80); 45% were female. Controls were more likely than case-patients to have treated their drinking water by boiling or using a chlorine product before the outbreak began in Port-au-Prince (matched odds ratio [mOR]=0.3; 95% confidence interval [CI] 0.1, 0.9), and to demonstrate proper handwashing technique (lathering hands with soap and drying with a clean cloth or air [mOR=0.2; 95% CI 0.03, 0.7]). Food exposures that were implicated as risk factors for transmission in previous cholera outbreaks were not associated with illness in this investigation. Our investigation demonstrated that personal hygiene measures taken by individuals and families, including treating drinking water and proper handwashing, helped protect against disease in this urban cholera outbreak. Improvements in access to water and sanitation infrastructure should be a high priority for government and aid organizations in post-earthquake Port-au-Prince to protect against cholera and other diarrheal disease outbreaks.

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MEMORY B CELL RESPONSES TO LPS ARE ASSOCIATED WITH PROTECTION AGAINST INFECTION IN HOUSEHOLD CONTACTS EXPOSED TO *VIBRIO CHOLERAE* O1

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Vibrio cholerae is a noninvasive enteric pathogen responsible for rapidly dehydrating diarrhea. Further understanding of the immune mechanisms mediating protection may be necessary for the development of a vaccine that confers protection comparable to natural infection. We have previously shown that memory B cells develop after cholera, and have hypothesized that these cells may play a role in long-term protective immunity. To test this hypothesis, we examined whether the presence in the circulation of memory B cells in individuals exposed to *V. cholerae* O1 in a household with a cholera patient was associated with protection from subsequent infection in contacts of the index case. We analyzed memory B cell responses to both the protein antigen cholera toxin B subunit (CTB) and the non-protein antigen lipopolysaccharide (LPS) in a cohort of 236 household contacts of 122 index cases. We also analyzed baseline vibriocidal and plasma antibody responses against CTB and LPS for correlation with protection. As previously described, we found that higher baseline vibriocidal antibody levels correlated with a decreased risk of subsequent infection in contacts ($P \leq 0.001$). The presence of LPS-specific IgG memory B cells on exposure conferred a 68% decrease in the subsequent risk of infection in household contacts ($P = 0.032$). No protection was provided by the presence of IgG or IgA memory B cells to CTB or IgA memory B cells to LPS. Previous studies have shown that LPS-specific IgG memory B cells decline to baseline levels within one year following cholera, although protection against subsequent infection persists for several years. It is possible that memory to LPS at the mucosal surface may last longer than in the circulation and may mediate protective immunity.

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HIGH-THROUGHPUT PROTEOMIC-BASED SCREENING OF ANTI- *VIBRIO CHOLERAE* ANTIBODY RESPONSES IN HUMANS

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Vibrio cholerae causes an estimated 3-5 million cases and 100,000 deaths, annually. Although current vaccines have been shown to be safe and immunogenic, none provide the long-lasting protective immune responses seen with natural infection. Characterization of immunogenic *V. cholerae* antigens could lead to a better understanding of protective immunity in human cholera infection. Using a high-throughput proteomic-based platform called the Nucleic Acid Programmable Protein Array (NAPPA), we screened 3,761 *V. cholerae* open reading frames (97% of the ORFeome) for anti-*V. cholerae* IgG responses in 25 cholera patients,10 vaccinees who received whole cell-killed vaccine with recombinant cholera toxin (WC-rBS), and 10 North American volunteers. In our primary screen of cholera patients, we detected significantly higher IgG reactivity in convalescent sera to over 300 proteins including a number of previously identified immunogenic and virulence-associated proteins (e.g. cholera toxin B, CtxB; toxin co-regulated pilus A, TcpA; *V. cholerae* cytolysin, VCC/hlyA) when compared to acute sera, healthy Bangladeshis (pre-vaccine) and/or North American volunteers. We also identified several proteins which annotate as methyl-accepting chemotaxis (e.g. VC1069, VC0514), flagellin proteins FlaB and FlaC, heat shock protein HtpX, and several hypothetical proteins. A subset of proteins had significantly increased IgG responses at convalescence from infection, but no increased IgG immunoreactivity after vaccination. This list included OmpW, FlaB, FlgJ, and EpsL, EpsG and EpsE. Outer membrane protein W has been shown to be immunogenic and antisera in rabbits have been to shown to be protective in a rabbit ileal loop model. FlaB and FlgJ encode a flagellin and flagellar protein, respectively. EpsL, EpsG, and EpsE encode general secretion pathway proteins which are involved with secretion of cholera toxin and other virulence factors. These findings give insight into differences in immune responses elicited after natural infection and vaccination, and may aid in the development of improved cholera vaccination approaches.

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THE CASE FOR REACTIVE MASS ORAL CHOLERA VACCINATIONS, A DUKORAL AND SHANCHOL MODEL

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The massive outbreak of cholera in Haiti intensified interest in the control and prevention of cholera. Momentum for the use of OCVs has been magnified by the licensing in India of a killed whole-cell (WC) oral cholera vaccine without the B-subunit (Shanchol). This vaccine is available at low cost, administration does not require a buffer solution, immunity is acquired one week after the first dose, and the protective efficacy (PE) last for at least 36 months. Datasets of cholera outbreaks from three sites with varying cholera endemicity Zimbabwe, Kolkata (India), and Zanzibar (Tanzania) were analysed to estimate the number of cholera cases preventable with reactive vaccination under differing response times, vaccine coverage, and vaccine doses. The PE assumptions for Shanchol were 67% for 36 months, starting 1 week after completion of the first dose. Assumptions for the recombinant B-subunit containing WC vaccine (Dukoral) were 85% PE for the first 6 months, 60% up to 18 months and 20% up to the 36 month, starting one week after completion of the second dose. During a large cholera outbreak in Zimbabwe in 2008/9, 98,591 cholera cases were reported with 4,288 deaths. If a rapid response had taken place and half the population were vaccinated as many as 33,122 (34%) cholera cases and 1,391 (32%) would have been prevented with Shanchol and as many as 34,900 (40%) cholera cases and 1,695 deaths (40%) with Dukoral. With a delayed response Shanchol would have prevented more cases than Dukoral. In the sites with endemic cholera, Kolkata and Zanzibar, a significant number of cases could have been prevented with either vaccine but the impact less dramatic. Shanchol would have prevented more cases if the outbreak was extended. If the major peak of the outbreak occurred immediately following vaccination Dukoral would have prevented a larger proportion of cases. Once a substantial proportion of a population is vaccinated outbreaks in subsequent years may be reduced if not prevented. We show that reactive mass vaccinations are a rational response to cholera outbreaks in endemic and non-endemic settings using either Shanchol or Dukoral. Decision makers in donor and recipient countries should be made aware of the potential benefit of reactive cholera vaccinations.

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A COMPARISON OF MEMORY B CELL, ANTIBODY SECRETING CELL, AND PLASMA ANTIBODY RESPONSES IN YOUNG CHILDREN, OLDER CHILDREN, AND ADULTS WITH INFECTION CAUSED BY *VIBRIO CHOLERAE* O1 EL TOR OGAWA IN BANGLADESH

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Children bear a large component of the global burden of cholera. Despite this, little is known about immune responses to cholera in children, especially those under five years of age. Cholera vaccine studies have demonstrated lower long-term protective efficacy in young children. Memory B cell (MBC) responses may correlate with duration of protection following infection and vaccination. Here we report a comparison of immune responses in young children (3-5 years of age; n=17), older children (6-17 years of age; n=17) and adults (18-60 years of age; n=68) hospitalized with cholera in Dhaka, Bangladesh. We found that while young children had lower baseline vibriocidal antibody titers than adults (P=0.02), they had higher fold-increases between day 2 and day 7 (P=0.04). Young children had higher baseline IgG plasma antibody levels to *Vibrio cholerae* antigens (P=0.03 for cholera toxin B, P=0.05 for lipopolysaccharide), although the magnitude of responses at day 7 and 30 were similar across age groups. As a surrogate marker for mucosal immune responses, we assessed day 7 antibody secreting cell (ASC) responses. These were comparable across age groups, although there was a trend for older age groups to have higher levels of lipopolysaccharide specific IgA ASC responses (P=0.07). All age groups developed comparable MBC responses at day 30 to *V. cholerae* lipopolysaccharide and cholera toxin B subunit. These findings suggest that despite some differences, young children are able to mount robust vibriocidal, plasma antibody, ASC, and MBC responses against *V. cholerae* O1, suggesting that under an optimal vaccination strategy, young children could achieve protective efficacy comparable to that induced in adults.

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UNIQUE APPROACH TO THE MANAGEMENT OF DELUSIONAL PARASITOSIS

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Delusional parasitosis (DP) is a fixed, false belief that one is infested with parasites, worms, bacteria, mites, or other living organisms. DP may be a manifestation of underlying medical or psychiatric disorders (secondary DP), or can be a primary delusional disorder. DP can be effectively treated by neuroleptic medications, but patients with DP will generally refuse psychiatric referral. We describe our systematic yet simple approach to treating patients with DP at the Tropical Disease Unit (TDU) at Toronto General Hospital and report our results. Our first visit consists of a thorough medical assessment of the patient, as well as complete laboratory investigations to rule out both true parasitic infection and treatable causes of secondary DP. At follow-up ≥ 4 weeks later, if all investigations are normal, we introduce the idea that symptoms are due to a "chemical imbalance" that may have resulted from a previous parasitic infection, and suggest the use of neuroleptic agents to "rebalance" the patient's chemistry. The records of 82 DP patients who were referred to and assessed at the TDU between 1/2005 and 12/2010 and followed for at least 4 months were assessed retrospectively. There were 33 (40%) males and 49 (60%) females, with a combined mean age at clinical presentation of 54.0 years (SD 11.6, range 24-85). Our approach led to 75 patients (91.5%) agreeing to try a neuroleptic. Of these patients, 38 (50.7%) showed some response: 11 (14.7%) had a complete response with full resolution of delusions, 19 (25.3%) had a major response, and 8 (10.6%) had a minor response; 14 (18.7%) did not respond. Seventeen (22.7%) were lost to follow up, and 6 (8.0%) were ultimately treated by another physician. The majority of patients who responded to therapy (32/38, 84%) were treated with risperidone. Patient acceptance of our "chemical imbalance" approach has been highly successful in our centre, although the effectiveness of therapy remains suboptimal.

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NEWLY RECOGNIZED CLINICAL SYNDROMES OF SNAKE BITE ENVENOMING

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Snake bites can no longer be dismissed as being too rare and inconsequential to deserve the time and attention of practitioners of tropical medicine. Recently published national epidemiological surveys estimated about 50,000 snake bite deaths each year (0.5% of all deaths) in India and 6,000 in Bangladesh. Even when geographically-appropriate polyspecific antivenom is available, species identification is crucial to allow anticipation, early treatment and prevention of life-threatening complications. Recognition of a characteristic clinical syndrome contributes to diagnosis when examination of the snake and rapid immunodiagnosis are impossible. However, the range of clinical features of envenoming associated with each taxon is broadening. Multi-focal thrombotic microangiopathy, causing cerebral and other infarctions, has been documented in victims of the two Lesser Antillean lanceheads, Asian Russell's vipers, some North American rattlesnakes, African puff adders and lowland vipers. In South Asian countries, kraits menace those sleeping on the floors of their homes. In the Indian sub-continent, severe abdominal pain is the dominant presenting symptom of envenoming by common kraits but its mechanism remains unknown. In Bangladesh, rhabdomyolysis leading to fatal acute kidney injury (AKI) was caused by bites of greater black kraits, a species previously unknown in that country. In Vietnam, patients envenomed by two species of krait developed fatal hyponatremia as well as hypertension, rhabdomyolysis and persistent mydriasis. Envenoming by some species of coral snakes in North and South America may produce unexpected effects including rhabdomyolysis, coagulopathy, gastro-intestinal and urinary tract bleeding, hemolysis and excruciating local pain. Atypical clinical presentations, without the expected paralysis, have been observed recently in victims of two classically neurotoxic species: severe local envenoming and coagulopathy caused by Eastern green mambas and rhabdomyolysis and AKI caused by smooth-scaled death adders in New Guinea. A hemolytic-uremic-syndrome-like picture of microangiopathic hemolysis with thrombocytopenia and AKI has been described with envenoming by some African and Indian Viperidae and Australian Elapidae. These findings demand radical revision of currently accepted concepts of the symptomatology associated with envenoming by various species of snakes.

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URINARY TRACT INFECTIONS AMONG PREGNANT WOMEN IN COAST PROVINCE, KENYA

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Urinary tract infection (UTI) is common in pregnancy, affecting 6-16% of women worldwide. Maternal pyelonephritis, low birth weight, and preterm birth are associated with UTI in pregnancy, so prompt diagnosis and treatment is essential. In much of the developing world, the rates of UTI and offending organisms are poorly described, as urine culture is not routinely performed. Our aim was to understand the profile of UTI during pregnancy in the region to inform diagnosis and treatment decisions. 159 expectant mothers were recruited at their first antenatal visit from March-May 2011 from the antenatal clinic at Msambweni District Hospital in Coast Province, Kenya. Mothers were asked about history of UTI and current UTI symptoms, and midstream clean-catch urine was collected. Urine was analyzed by dipstick, microscopy, and urine culture. Antibiotic sensitivities were determined by Kirby-Bauer method. Of 159 women, 29 (18.2%) had significant bacteriuria (>10^5 CFU/ml), and another 18 (11.3%) had a lesser degree of bacteriuria (>10^4 CFU/ml). Thirty-eight percent (38%) of recruited women had UTI symptoms, and 17% had a history of UTI. The most frequently isolated bacteria were *S. aureus* (46% of positive cultures), *Enterococcus* (21%), *E. coli* (16%), and *Klebsiella* spp. (12%), which demonstrated 4%, 40%, 38%, and 33% resistance, respectively, to ampicillin, the most commonly prescribed antibiotic. Other pathogens detected on microscopy included *Schistosoma haematobium* (6.4% of women), *T. vaginalis* (3.6%), and *Candida* spp. (6.4%). A combination of dipstick (nitrite and leukocyte esterase (at least 2+)) and microscopy (>5 leukocytes/hpf) was 75% sensitive and 43% specific for detecting bacterial and non-bacterial UTIs. Our findings suggest that levels of UTI among pregnant women in our study population are somewhat higher than rates recorded in other developing countries, and *S. aureus* bacteriuria comprises a higher percentage of infections than previously reported in the literature. The current screening method (dipstick + microscopy) is relatively sensitive for detection of UTIs, but it lacks specificity, leading to unnecessary antibiotic usage and possibly increased antibiotic resistance. Urine culture with moderately priced media (i.e. CLED agar) should be considered to confirm positive UTI screens, minimizing the sequelae of UTI in pregnancy and the development of bacterial antibiotic resistance.

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A NOVEL BUBBLE CPAP DEVICE FOR LOW RESOURCE SETTINGS

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Respiratory distress and failure are leading causes of infant morbidity and mortality in the developing world. Infants who are in respiratory distress can be supported with methods of non-invasive ventilation such as bubble Continuous Positive Airway Pressure (bCPAP); however, bCPAP devices are not readily available in low resource settings due to high cost -- on average, $6,000 -- and technical complexity. To address this need, we engineered a novel bCPAP system which can be made at a unit cost of only $160. Moreover, because of its simple design, it requires only the replacement of a $1 diaphragm approximately every 2 years to maintain. The novel bCPAP device consists of an adjustable flow generator and a pressure-regulated delivery system. The adjustable flow generator, consisting of two diaphragm pumps, provides a continuous flow of ambient air that is controlled with a standard flow regulator. If supplemental oxygen is required, the output of an oxygen concentrator or tank can be connected to an input port in the flow generator. By adjusting the flow of ambient air and oxygen, the % oxygen administered can be controlled. The pressure-regulated delivery system, consisting of nasal prongs and a pressure control tube submerged in a column of water, controls the air pressure that is delivered. The objective of this study was to evaluate the output pressure of this novel bCPAP device. The nasal prong pressures of the novel bCPAP device and a comparable, bCPAP system at the Texas Children's Hospital (TCH) were measured using a digital pressure sensor and collected continuously over a 2-minute period. At a flow setting of 7 L/min and a water column level of 6cm, nasal prong pressure data of the novel bCPAP device and of the TCH bCPAP system indicate equivalent output pressure ranges. The novel bCPAP device provides a method for low-cost, easy-to-use and repair respiratory support and has the potential to successfully treat respiratory distress in infants and small children in low resource settings.

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TRAVEL-RELATED ILLNESSES AMONG PEDIATRIC TRAVELERS WHO VISIT FRIENDS AND RELATIVES (VFRS) IN CANADA

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Travelers who visit friends and relatives (VFRs) are at increased risk of travel-related illnesses (TRIs); however, there is little data regarding travel-related illnesses among pediatric VFRs. The Canadian Paediatric Surveillance Program (CPSP) is an active national surveillance program that collects data from approximately 2,500 pediatricians and pediatric sub-specialists in Canada. We undertook a two year surveillance project through the CPSP of all cases of significant travel-related illness among pediatric VFRs in Canada from March 2009 to February 2011. Mild respiratory and gastrointestinal illnesses were excluded. There were 88 confirmed cases of significant travel-related illnesses among pediatric VFRs in Canada. Among the pediatric TRIs, 64% were acquired in Asia, 21% in Africa, 12% in Central/South America and the Caribbean, 2% in the Middle East and 1% in Europe. The average duration of travel was 7 ½ weeks. Enteric fever (31 confirmed and 5 presumed cases) was the most common TRI. Malaria (17 cases) and hepatitis A (11 cases) were the next most common TRIs. Fever was the most common presenting symptom in 80% of children with TRIs. Three patients presented with hypotension (malaria) or septic shock (Salmonella bacteremia). There was one death and four children had significant sequelae following their TRIs. Three quarters of cases required hospitalization (n=67) with an average length of stay of 11 days (median 5.5 days). The majority of pediatric VFR travelers did not seek pre-travel advice (73%) and among those who did, only 1/3 sought advice from a travel health clinic. Our data indicate that TRIs cause significant morbidity and some mortality among pediatric VFRs in Canada. Furthermore, the majority of the TRIs were potentially preventable if appropriate pre-travel advice had been obtained and followed. This highlights the need for increased education of families and health care providers regarding the importance of pre-travel advice to minimize the risk of acquiring travel-related illnesses among pediatric VFRs.

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PREDICTING ADVERSE OUTCOMES AMONG PEDIATRIC INPATIENTS IN AN AREA OF LOW MALARIA TRANSMISSION IN TANZANIA

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The proportion of infants and children living in areas of low malaria transmission intensity in sub-Saharan Africa is increasing. To improve child health outcomes, methods for identifying severe illness need to be evaluated in areas where malaria is uncommon. We identified febrile pediatric patients among consecutive admissions in Moshi, Tanzania from September 2007 to August 2008, recorded clinical data using Integrated Management of Childhood Illness (IMCI) criteria, and collected diagnostic specimens. Of 466 participants with known hospital outcomes, median age was 1.4 years (range 2 months-13 years), 266 (57.0%) were male, 11 (2.4%) had malaria, and 34 (7.3%) died. Inpatient death was associated with Blantyre coma score <5 (OR 23.2, p<0.001); central cyanosis (OR 20.6, p=0.003); capillary refill >3 seconds (OR 9.0, p<0.001); inability to breastfeed/drink (OR 8.9, p<0.001); stiff neck (OR 7.0, p<0.001); bulging fontanelle (OR 6.5, p=0.024); severe anemia (OR 5.3, p<0.001); lethargy (OR 5.2, p<0.001); severe wasting (OR 4.9, p <0.001); skin pinch >2 seconds (OR 4.8, p=0.003); abnormal breath sounds (OR 4.3, p<0.001); signs of respiratory difficulty (OR 4.0, p<0.001); history of fever >7 days (OR 3.8, p<0.001); generalized lymphadenopathy (OR 3.6, p=0.005); oral candidiasis (OR 3.4, p=0.015); history of convulsions in past 48 hours (OR 2.8, p=0.017); referral from another inpatient facility (OR 2.5, p=0.010); low mid-upper arm circumference aged ≥6 to <60 months (p=0.003); and low weight-for-age Z score (p=0.014). Factors not associated with death included: hypoglycemia (OR 6.4, p=0.206); hepatomegaly (OR 2.5, p=0.068); severe pallor (OR 1.8, p=0.288); splenomegaly (OR 1.5, p=0.517); jaundice (OR undefined, p=1.00); and positive malaria film (OR undefined, p=1.00). Overall IMCI criteria performed well for predicting in-hospital death although typical malarial signs had little value. Health care workers should be trained to recognize differences in the constellations of clinical signs associated with severe illness in an area of low malaria transmission.

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THE CONTRIBUTION OF MALARIA RETINOPATHY TO REDUCING MORTALITY FROM *PLASMODIUM FALCIPARUM* MALARIA IN ASIA

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The treated mortality of severe malaria remains high. Adjunctive therapies aiming to reduce this should target pivotal aspects of its pathogenesis. However, understanding of the pathogenesis is incomplete and there is a lack of surrogate markers for use as endpoints in treatment trials. Management is hampered by difficulty distinguishing malarial from non-malarial coma and predicting prognosis. Detailed description of malaria retinopathy can address many of these issues. We describe a series of observational studies of malaria retinopathy in Bangladesh and India in 2008-2011. The aims were to assess the potential contribution of retinopathy to diagnosis of malarial coma, assessing prognosis, understanding pathogenesis and as a surrogate marker for adjunctive treatment studies. Admitted smear positive patients with *P. falciparum* malaria of any severity plus control groups of febrile encephalopathy, sepsis and healthy volunteers were recruited. Patients' retinas were photographed daily until discharge then weekly until normal. Detailed clinical assessment, markers of and contributors to microcirculatory obstruction (plasma lactate, rectal capillary blood flow, PfHRP2 and red blood cell stiffness), fluorescein angiography and cerebral MRI were performed. 287 patients were enrolled (192 with malaria, and 30 in each control group). Retinopathy was most common and severe in cerebral (85%) and fatal (90%) malaria and correlated with severity of malaria. It was specific for cerebral malaria in comatose patients (94%), and its severity correlated with coma recovery time. It resolved in 2 weeks but visual function recovered in 3-4 days. Malaria retinopathy correlated with underperfusion and blood retinal barrier (BRB) leakage on angiography; plasma lactate, red cell stiffness and PfHRP2-derived parasite biomass. Malaria retinopathy is a bedside tool which can help diagnosis and prognosis in patients with cerebral malaria. Retinopathy is caused by microvascular obstruction and BRB leakage and by serial assessment can be used as a surrogate marker for intervention studies.

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ENDOCRINE PANCREAS AND *TRYPANOSOMA CRUZI* - A LINK TO DIABETES?

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Trypanosoma cruzi infection (Chagas disease) affects millions of people in South and Central America. Recently, Chagas disease has become a public health concern in non-endemic areas including the United States due to immigration. So far researches have been focused on the studies on *T. cruzi*-induced cardiomyopathy and digestive isorders. Here in for the first time we have shown that *T. cruzi* infection may have a direct link to insulin secretion and metabolic disorders. CD1 mice infected with Brazil strain trypomastigotes had a significant decrease in insulin levels (3.5 fold) compared with uninfected mice. Reduced levels of insulin persist into the chronic stage (day 133 post infection). We measured Insulin response to the administration of L-arginine in infected and infected mice. L-arginine stimulated acute phase of insulin secretion in uninfected mice

but the response in infected mice was significantly reduced in acute and chronically infected mice. Administration of the beta-3 adrenergic receptor agonist (CL316,243) increased plasma insulin levels (4 fold) in uninfected mice but the response in infected mice was reduced. This was observed in acute and chronically infected mice. Histopathological studies displayed the presence of parasites in the acinar cells of pancreas during acute infection. H&E staining of the pancreas revealed a massive inflammation during acute infection which persisted into the chronic phase. The pancreas synthesizes several hormones including insulin, glucagons and somatostatin thereby governing carbohydrate and lipid metabolism. Malfunctioning of pancreas during *T. cruzi* infection may cause insulin resistance and diabetes.

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IFN- Γ REDUCES CELLULAR IRON INTAKE THROUGH REGULATION OF IRP2 EXPRESSION, A WAY TO CONTROL *LEISHMANIA INFANTUM CHAGASI* REPLICATION IN MACROPHAGES

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Visceral leishmaniasis (VL) is endemic in Brazil and *Leishmania infantum chagasi* is the main etiological agent. Dogs are the main domestic reservoir of leishmania and, like humans, develop a spectrum of clinical forms that varies from asymptomatic to severe and fatal disease. When there is intracellular iron depletion, iron regulatory protein 2 (IRP2) binds to transferrin receptor mRNA, allowing its translation, which results in increased iron intake by the cell. *L. i. chagasi* infected cells tend to be depleted of iron. Our hypothesis is that iron scavenging is impaired by IFN-γ which limits *Leishmania* intracellular proliferation. Blood and spleen fragments were collected from euthanized dogs with VL (n=31). qPCR was used to assess IRP2, IFN-γ and IL-10 gene expression in spleen RNA. Parasite load was evaluated in spleen by qPCR for kDNA. ANOVA test followed by Tukey post-test was used to assess differences among the groups and Spearman test was performed with a significance of 5%. Dogs were grouped by parasite load: Group 1 (n=6), <1,000 parasites/spleen µg; Group 2 (n=7)1,000-10,000; Group 3 (n=6) 10,000-100,000 and group 4 (n=12) >100,000. Analysis was performed comparing group 1 with the other groups. This study was approved by Ethical Committee for Animal Research of Federal University of Rio Grande do Norte. IRP2 expression in groups 2 and 3 was increased two (p=0.0162) and four fold (p=0.0066), respectively, whereas IFN-γ decreased 4 and 5 fold in the same groups (p=0.0385; p=0.0195). An inverse correlation between IFN-γ and IRP-2 was observed (r=-0.906). Conversely, IL-10 was directly correlated with the parasite load (r=0.577), in groups 2 (p=0.0095) and 3 (p=0.0071). Spleen cells from dogs with lower leishmania load presented higher expression of IFN-γ and decreased expression of IRP2. Thus, IFN-γ-induced iron depletion in the macrophage may be a mechanism for control *L.i.chagasi* replication by host cells.

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B7-1/B7-2:CD28/CTLA-4 COSTIMULATION PLAYS A CRITICAL ROLE IN ESTABLISHMENT OF CHRONIC *LEISHMANIA MEXICANA* INFECTION IN C57BL6 MICE

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Using mice deficient in B7-1, B7-2 or both molecules, we had previously shown that B7-2 mediated pathway induces IL-4 production and plays a role in pathogenesis of *L. major* infection in BALB/c mice. However, the role of B7-1/B7-2:CD28/CTLA4 co-stimulation pathway in infections caused by other *Leishmania* species such as *L. mexicana* is not clear. In this study, we analyzed the role of B7/CD28 family molecules in *L. mexicana* infection by monitoring cutaneous growth of *L. mexicana* in B7-1$^{-/-}$/B7-2$^{-/-}$, CD28$^{-/-}$/CTLA4$^{-/-}$, PDL1$^{-/-}$, PDL1$^{-/-}$/CD28$^{-/-}$/CTLA4$^{-/-}$ and wild type (WT) C57BL6 mice. Following s.c. inoculation with *L mexicana* amastigotes into back rump, B7-1$^{-/-}$/B7-2$^{-/-}$ mice developed no lesions or significantly smaller lesions containing significantly fewer parasites compared to similarly infected WT mice. CD28$^{-/-}$/CTLA4$^{-/-}$ mice developed lesions similar to WT mice during early course of infection but resolved them eventually. Interestingly, CD28$^{-/-}$/CTLA4$^{-/-}$ mice lacking PDL1 (PDL1$^{-/-}$/CD28$^{-/-}$/CTLA4$^{-/-}$) were highly susceptible to *L. mexicana* infection similar to WT mice and PDL1$^{-/-}$ mice. Unlike the WT mice which had high serum titers of parasite specific IgG1 both the B7-1$^{-/-}$/B7-2$^{-/-}$ and CD28$^{-/-}$/CTLA4$^{-/-}$ C57BL6 mice did not elicit significant antibody titers during the course of infection. At week 13 post-infection, *L. mexicana* antigen-stimulated lymph node cells from CD28$^{-/-}$/CTLA4$^{-/-}$ mice produced more Th1 associated IL-12 and IFN-γ compared to similarly stimulated lymph node cells from WT mice which produced significantly higher levels of Th2-associated IL-4. Lymph node cells from PDL1$^{-/-}$/CD28$^{-/-}$/CTLA4$^{-/-}$ mice did not elicit significant levels of IFN-γ, IL-12 nor IL-4 at this time point. Taken together, these results indicate that B7-1/B7-2:CD28/CTLA-4 interaction plays a critical role in pathogenesis of cutaneous leishmaniasis caused by *L. mexicana* by inducing disease exacerbating Th2 response and IL-4 production. Furthermore, our findings suggest that PDL1 associated pathway contributes to enhanced resistance of CD28$^{-/-}$/CTLA4$^{-/-}$ mice against *L. mexicana*.

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SARCOCYSTIS SPECIES IN MALAYSIA: A MOLECULAR CHARACTERIZATION USING DNA PROFILLING

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This study was carried to establish the DNA profile of *Sarcocystis* species found in wild rodents in Peninsular Malaysia. One hundred and forty six rodents belonging to 7 species trapped in the states of Johor, Selangor, Kelantan and Kedah were examined. Rodents as an intermediate host to *Sarcocystis* pose a public health. Studies have shown the prevalence rate of *Sarcocystis* in Southeast Asia to be high. Human infections with Sarcocystis spp. from rodents results in human muscular sarcocystosis, implicated with myalgia, erythematous subcutaneous nodules, fever, bronchospasm, cough, headaches, loss of appetite, weight loss and lethargy. Hematoxylin and eosin (H&E) stained sections of the tissues from the wild rodents showed the presence of *Sarcocystis* species. In the study using light microscopy, a total of 146 thigh muscles were examined and 73 (50%) were found to be positive. Morphological observation showed that there may be 3 different species infecting these rodents. The brain sections were found not to contain any cysts. To identify the species present in these wild rodents, DNA extraction was carried out on paraffin embedded blocks of tissue using 5 prime archive pure DNA cell/tissue kit. DNA profiling was done for the identification of the different species. The results of the analysis will be reported at the

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IMPORTANCE OF UBIQUITIN AND UBIQUITIN LIKE PROTEIN MODIFIERS (UFM1) CONJUGATION IN THE PATHOGENESIS OF *LEISHMANIA DONOVANI*

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Leishmaniasis is a spectrum of diseases caused by protozoan parasites belonging to several different *Leishmania* species. There are no effective vaccines against leishmaniases. Currently available therapeutic regimens are often limited in effectiveness due to unwarranted side effects and

rapidly emerging drug resistance. Therefore, the quest for a novel vaccine and therapeutic targets acquires urgency towards controlling leishmaniases. Ubiquitin and ubiquitin like protein modifiers (Ubls) regulate a variety of biological functions ranging from endocytosis, membrane trafficking, protein kinase activation, DNA repair and chromatin dynamics. Studies of Ubl functions in human parasitic organisms are limited. Recently, we described the existence of a novel Ubl named ubiquitin-fold modifier 1 (Ufm1) that conjugates to parasite proteins in *Leishmania donovani*. Our studies showed that the Ufm1 conjugation system is mitochondria associated and conjugation of Ufm1 to mitochondrial proteins occurs in a parasite stage-specific manner. We also showed that modification/alteration of proteins that regulate Ufm1 conjugation reaction results in reduced survival of *L. donovani* in infected human macrophages suggesting their role in *Leishmania* pathogenesis. In order to further elucidate the biological roles of the Ufm1-modification, we prepared an Ufm1 null mutant (ufm1-/-). Consistent with the earlier results, the ufm1-/-mutants showed reduced survival in amastigote stage and this defect was reversed by re-expression of wild type but not the non-conjugatable Ufm1 indicating the essential nature of Ufm1 conjugation reactions. Results including ultra-structural studies and the effects of absence of Ufm1 on the cell cycle regulation in *L. donovani* will be discussed. Further, ufm1-/- parasites also provide an opportunity to explore such parasites as live attenuated vaccine candidates.

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CHARACTERIZATION OF A NOVEL INVARIANT METACYCLIC SURFACE PROTEIN OF *TRYPANOSOMA BRUCEI BRUCEI*

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African trypanosomes of the *Trypanosoma brucei* complex undergo several differentiation steps during development in the tsetse fly, including differentiation to the procyclic form in the fly midgut, epimastigote forms in the proventriculus, and ultimately to mammalian infective metacyclic trypomastigotes in the salivary glands. Finally, after inoculation into the vertebrate host, metacyclic parasites develop into the bloodstream form. A recent in-silico screen of the *T. brucei* genome yielded 111 unknown proteins containing glycosylphosphatidylinisotol (GPI) anchor structures. GPI anchors typically bind proteins to cell membranes and as such, these hypothetical proteins may be expressed on the parasite cell surface. A semi-quantitative gene expression analysis performed on parasite infected salivary glands, proventriculus, and midguts, as well as bloodstream parasites looking for preferential expression in particular developmental stages was recently performed. This analysis revealed a trypanosome gene family specifically upregulated in infected salivary glands. Importantly, these genes were found to be expressed in the mammalian infective metacyclic form collected from tsetse saliva. This gene family was further characterization at the RNA and protein level in both tsetse fly, and mammalian host immediately after transmission. These data support the possibility of identifying novel transmission-blocking targets from investigations into tsetse salivary gland trypanosome stages. Importantly, these data shed light on the parasite biology immediately after transmission to the vertebrate host.

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IMMUNOLOGICAL DETERMINANT UNDERLYING THE CONTROL OF INFECTION IN HUMANS INFECTED BY *TRYPANOSOMA BRUCEI GAMBIENSE*

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Human African Trypanosomiasis or sleeping sickness is caused by *Trypanosoma brucei gambiense* and *T. b. rodhesiense* parasites that are transmitted to humans by tsetse flies. As for many infectious diseases it is now clear that a wide range of outcome may result from the infection by trypanosomes. The disease is classically characterised by an early haemolymphatic phase (stage 1) followed by a meningoencephalitic phase (stage 2) leading to neurological disorders and death if left untreated. However, in *T.b. gambiense* endemic area where mass screening of the population is routinely performed by the Card Agglutination Test for Trypanosomiasis (CATT), a high proportion of individuals displaying positive serological results are negative to direct parasitological investigations. Increasing evidence now indicate that at least part of these subjects are infected but harbour parasitaemia levels that are below the detection limit of the parasitological tests used in the field, suggesting that they are able to control infection. The nature of the immune response in these individuals has yet received poor attention. In this communication we report on the quantification of the cytokine levels (IL-12, IL-2, IL-4, IL-5, TNF-α, INF-γ, IL-8, IL-1β, IL-6, IL-10) measured in healthy endemic controls, stage 1 and stage 2 patients and on a cohort of seropositive subjects from Guinea that were followed up in time to assess the evolution of their parasitological status. Whereas HAT patients were characterized by elevated levels of IL-1β and IL-10, seropositive subjects exhibited high levels of IL-6, IL-8 and TNF-α and low levels of IL-1β, IL-12 and IL-10. Interestingly high levels of IL-10 in seropositive subjects were also associated with an increased risk of developing the disease in this category of subjects.

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A NOVEL, FULLY AUTOMATED SAMPLE-TO-RESULT REAL-TIME PCR ASSAY FOR POINT-OF-CARE DETECTION OF DENGUE VIREMIA

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Appropriate treatment of patients with dengue relies on early clinical recognition. Laboratory methods to confirm dengue virus (DENV) infection are time-consuming, labor-intensive, and require a high level of technical skill. RT-PCR, the most sensitive method for DENV detection, has been difficult to transfer to the clinical setting; the lack of integration and automation of nucleic acid tests has been one major obstacle. We have developed a fully-automated, rapid qualitative RT-PCR assay for the detection of dengue viremia based on IQuum's lab-in-a-tube (Liat™) platform, which integrates raw sample processing and detection, including target enrichment, inhibitor removal, nucleic acid extraction, reverse transcription and real-time PCR, in a single closed-tube format with a turnaround time of <35 minutes. We tested the performance of the assay using serum from known DENV-infected subjects collected as part of a

passive dengue surveillance program, as well as archived serum from healthy North American donors enrolled in two separate non-flavivirus vaccine studies. Serum vials were thawed and 150 µl per sample were extracted and run on the Liat platform. Assay results were compared to those using a published benchtop real-time RT-PCR method after RNA extraction. In comparison to the reference benchtop PCR assay, the Liat Assay detected DENV RNA in 46 of 46 PCR-positive samples, as well as in 8 of 10 samples with equivocal results (including 4 of 4 samples from IgM+ subjects). The Liat results were negative in all 6 samples where the reference assay yielded unequivocal negative results, and in all 31 samples from the North American study volunteers. More testing of archived and live samples is in progress, yet the preliminary testing of the Liat Dengue Assay suggests a high degree of sensitivity and specificity compared to traditional laboratory-based methods for detection of dengue viremia. When this is confirmed, the ease-of-use and fast speed of this Liat test will enable greater access to nucleic acid testing in decentralized settings.

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PERMISSIVENESS OF BONE MARROW PROGENITOR CELLS FOR DENGUE VIRUS INFECTION

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Dengue is one of the most important mosquito-borne viral diseases affecting humans, with over half of the world's population living in areas at risk. Bone marrow suppression associated with reduction of megakaryocytes has been observed in dengue patients during the acute stage of infection. Studies of bone marrow biopsies from patients during acute infection indicate dengue virus infection induces hypocellularity in bone marrow progenitor cells. *In vitro* investigations also revealed that bone marrow cells were highly permissive for dengue virus infection. Results from early attempts to investigate the possible underlying mechanisms leading to bone marrow suppression have been inconclusive. A systematic investigation on this subject was performed with bone marrow from 20 healthy rhesus monkeys. Freshly collected bone marrow aspirates were infected with low dose (MOI=0.1) of dengue virus, strain 16881 grown in Vero cells. Cell smears were performed and supernatant fluids collected daily for 10 consecutive days or on days 1, 2, 3, 5, 7, 10, and 14 after infection. NS1 concentration was quantified by ELISA and viral titers were measured by quantitative real-time RT-PCR. Supernatants obtained on days 2 and 5 were used to co-culture with Vero cells to evaluate the infectivity of the virus. Immunohistochemical staining with antibodies for cell surface markers and dengue viral antigen was performed on smears. Electron microscopy was used to evaluate viral particle in infected BM cells. Results revealed that bone marrow cells were i) highly permissive for dengue virus infection, especially from young monkeys; ii)the peak in viral titers were observed on day 3 after infection, which corresponded to the peak in NS1 concentration; and iii) infectious virus could be recovered predominantly from day 2 but less often from day 5. Surface marker staining of sequential daily samples indicated that progenitor cells expressing CD41 markers were positive for dengue viral antigen early (1-3 days) post infection, while cells with markers typical for dendritic cells or macrophages were positive for dengue viral antigen at later time periods (days 5-8) of infection. The significance of the findings will be discussed.

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DEVELOPMENT OF A RAPID, SPECIFIC AND SENSITIVE PCR-MICROSPHERE BEAD ASSAY FOR DIFFERENTIAL DETECTION OF DENGUE VIRUS SEROTYPES

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Differential diagnosis of dengue viruses (DENV) is typically conducted using the plaque reduction neutralization test (PRNT) or virus isolation followed by serotype-specific immunofluorescence assay, real-time RT-PCR, or sequencing. Although, PRNT, RT-PCR and sequencing are specific, they require several hours to days to obtain reliable results. Therefore, to provide rapid diagnosis of DENV infection, we developed a differential multiplex PCR-microsphere bead assay (PCR-MBA) assay to detect DENV-1, -2, -3, and -4 serotype-specific RNA. The PCR-MBA employs pan-flavivirus primers, a one step RT-PCR, serotype- and virus-specific probes, and the Luminex platform to differentiate between DENV-1 to -4 serotypes and five other related flaviviruses. The assay was validated for DENV using qRT-PCR, virus isolation, sequencing, and/or PRNT using 85 well-characterized serum specimens obtained from patients and healthy controls with wide age range and geographical distribution. The DENV PCR-MBA successfully identified 85 serum specimens (100%) with differential detection of DENV-1, -2, -3, and -4. Results for the remaining five arboviruses were in accordance with the serum panel and were negative with low background values. Based on analysis of 85 serum specimens, the specificity, sensitivity, accuracy and negative predictive value for the PCR-MBA was 100% when compared to in-house confirmatory tests; qRT-PCR, RT-PCR, virus isolation, PRNT, and sequencing. These data indicate that the DENV PCR-MBA is both sensitive and specific when tested using a panel of DENV-1, -2, -3 and -4 positive serum samples and a large panel of negative controls. The primary advantage of the PCR-MBA reported here is the rapid detection of DENV, multiplex detection of DENV serotypes circumventing immunofluorescence assay and PRNT for DENV serotyping, ability to concurrently test up to 96 samples, and use of the Luminex platform that provides a high degree of automation and standardization. This assay has potential to be employed for simultaneous detection of other related arboviruses.

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EVASION STRATEGIES AND EARLY DETECTION OF DENGUE VIRUS (DENV) BY PRIMARY HUMAN DENDRITIC CELLS

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Production of type I interferon (IFN), is an essential first engagement of the host innate immunity for the control of many viral infections. The induction of these fundamental cytokines is initiated upon detection of virus by the cellular pattern recognition receptors (PRRs). Dengue Virus (DENV), the most prevalent arbovirus in humans, can modulate the host immune response using both, passive escape strategies, such as hiding its replication products from the host PRRs, and also in an active fashion, expressing factors that antagonize the cellular innate immunity. The main mechanism of immune evasion by DENV, described by several groups, is the interference of the type I IFN signaling pathway. Recently our laboratory reported the inhibition of type I IFN production in human monocyte derived DCs (mDCs), with an otherwise strong cytokine and chemokine profile in those cells. On a subsequent report we demonstrated that the NS2B3 protease complex of DENV, functions as an antagonist of type I IFN production in mDCs, and its proteolytic activity is necessary for this function. We are currently performing target pull down experiments using the tandem affinity purification strategy with different versions of the protease complex (NS2B3) as a bait to further identify possible

cellular targets and characterize the mechanism of inhibition of Type I IFN production in human immune cells by this viral product. Additionally, we are analyzing the immune response in human DCs, using primary isolates of DENV and a series of deletion mutants of the 3' untranslated (UTR) region. Our preliminary published results show that specific deletions in this region, previously shown to affect replication in some mammalian cells may also confer a rearrangement of the viral RNA structures that could be sensed differently by the cellular PRRs, as described for other pathogens.

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DENGUE VIRUS TRANSMISSION VIA MICROPARTICLES SHED FROM INFECTED MEGAKARYOCYTIC AND ERYTHROCYTIC PROGENITOR CELLS

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Dengue Virus is one of the most important vector-borne human pathogens, causing the most mosquito-transmitted infections, leading to 50-100 million hospitalizations a year. Disease may manifest in many forms, ranging from asymptomatic to life-threatening illnesses, such as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). One key characteristic of dengue disease in humans is the presence of viremia, which may last as long as seven days. The patient viral load, measured by qRT-PCR, may reach as high as log 9 genome equivalents per ml of whole blood. Despite this high level of infectious virus, there are virtually no detectable classical viral particles that can be visualized by electron microscopy (EM). Recent evidence from our lab has shown virus like particles inside of platelets and the presence of free vesicles concentrated from the serum of infected patients. This work suggests that the morphology of virus propagated in humans differs from the structures observed from cell culture systems, such as Vero. Our investigation has extended to *in vitro* dengue virus infections with the megakaryocytic and erythrocytic progenitor cell lines, K562 and Meg-01. EM results demonstrate that the morphology of virus in the supernatant from these cells are vesicle-like, consistent with that observed from human patient serum. Transmission EM analysis demonstrates the presence of dengue envelope positive membrane vesicles, or DV-MPs, from the supernatants of virus-infected cells. The DV-MPs from these cells are positive for dengue virus by fluorescent cell staining. Radio-labeling experiments show that serum from convalescent dengue patients can recognize a unique 55kDa protein from DV-MPs derived from infected K562 and Meg-01. Infectious virus can be recovered from the DV-MPs isolated from these cell lines as well as from dengue patients by co-culture with Vero cells. This data suggests that dengue virus may be disseminated *in vivo* through vesicle membranes shed from the surface of infected cells.

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BIOMARKERS PREDICT PROGRESSION OF UNCOMPLICATED DENGUE FEVER TO DENGUE HEMORRHAGIC FEVER IN BUCARAMANGA, COLOMBIA

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Dengue represents the most important arboviral infection worldwide and is of increasing global importance. The development of hemorrhagic manifestations in dengue fever is associated with increased mortality. Therefore, biomarkers that improve prediction of individuals in need of referral and hospitalization could lead to better clinical decision making and reduced mortality. This study was undertaken to determine if i) perturbations in host biomarkers from pathways of known pathogenesis in dengue fever can identify individuals at-risk of clinical deterioration; and ii) host biomarkers, when combined with useful clinical parameters, can improve clinical assessment of dengue patients early in disease. Using a case-control design, we randomly selected subjects from a prospective cohort study of acute dengue in Bucaramanga, Colombia. Using serum collected from subjects within the first 96 hours of illness, we tested 18 biomarkers by ELISA in cases (DHF, n=46; subjects with dengue fever who developed hemorrhagic manifestations) compared to controls (DF, n=66; subjects with uncomplicated dengue fever). sICAM-1, sEndoglin and IP-10 were elevated in subjects who developed DHF (p=0.009, p=0.022 and p=0.014 respectively). In a logistic regression model, age (odds ratio (OR), (95% CI): 0.92 (0.92-0.98), p=0.001), burning skin (OR; 3.7 (1.3-10.2), p=0.012) and elevated sICAM-1 (>298ng/mL: OR; 7.4 (2.1-26.0), p=0.002) were independent predictors of hemorrhage. We asked whether sICAM-1 improved prediction by comparing the c-indices from logistic regression models of clinical parameters with or without sICAM-1. The model with sICAM-1 had superior predictive ability (c-index of 0.83 vs. 0.74, p=0.013 by Delong et al.). Using a classification approach (CRT), elevated sICAM-1 (>300ng/mL) followed by age (<35 years) were the best predictors of progression to DHF. In conclusion, these data suggest sICAM-1 may be a clinically useful biomarker to predict complications in dengue fever and may point to endothelial activation as a critical pathway in dengue pathogenesis.

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INTRAHEPATIC INFILTRATING CELLS CAUSE LIVER CELL DEATH IN DENGUE VIRUS INFECTION

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Lymphocyte activation, hepatic infiltration and elevated liver enzyme levels are observed in dengue patients. However, the pathogenic mechanism of liver damage has not been carefully studied. The aim of this study was to investigate the pathogenic mechanism of liver injury in dengue. We have previously shown that immunocompetent C57BL/6 mice infected intravenously by dengue virus have transient elevation of liver enzymes AST and ALT. Employing this model we investigated intrahepatic cellular infiltration and its relationship to liver cell death. Our results showed that dengue virus infection induced CXCL9 and CXCL10 expression in the liver. There was a peak of NK cell infiltration at day 1 after infection. Depleting NK cells reduced the cleaved-form of caspase 3 and the number of $TUNEL^+$ cells in the liver. In addition, following the expression of CCL5, $CD4^+$ T and $CD8^+$ T cell infiltration peaked at day 5 and the infiltrating cells were cytotoxic against dengue virus-infected Hepa 1-6 targets. TCRβ deficiency or $CD8^+$ T cell depletion reduced cleaved-form of caspase-3 and $TUNEL^+$ cells in the liver. Interestingly, intrahepatic $CD8^+$ T cells as well as splenic $CD8^+$ T cells recognized DENV $NS4B_{99}$ as a dominant peptide. These results together show that liver cell death at early time point after infection is related to NK cell infiltration and that at later time point is caused by specific $CD8^+$ T cells cytotoxicity, possibly those that recognize $NS4B_{99}$.

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TREATMENT GUIDED BY RAPID DIAGNOSTIC TESTS FOR MALARIA IN TANZANIAN CHILDREN; SAFETY AND TREATABLE ALTERNATIVE DIAGNOSES TO MALARIA

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WHO guidelines for the treatment of young children with suspected malaria have recently changed from presumptive treatment for malaria to anti-malarial treatment guided by a blood slide or malaria rapid diagnostic

test (RDT). However, there is limited evidence of the safety of this policy in routine outpatient settings in Africa. Children 3-59 months with a non-severe febrile illness and no obvious cause were enrolled over 1 year period in a malaria endemic area of Tanzania. Treatment was determined by the results of a clinical examination and RDT, and blood culture and serum lactates were also collected. RDT-negative children were followed up over 14 days. Overall, 965 children were enrolled; 158(16.4%) were RDT-positive and treated with artemether-lumefantrine (ALu) and 807(83.4%) were RDT-negative and treated with non anti-malarial medicines. Compared with RDT-positives, RDT-negative children were on average younger with a lower axillary temperature and more likely to have a history of cough or difficulty in breathing. Six (0.6%) children became RDT-positive after enrolment, all of whom were PCR-negative for *P. falciparum* DNA at enrolment. In addition, 12 (1.2%) children were admitted to hospital, one with possible malaria, none of whom died. A bacterial pathogen was identified in 9/965 (0.9%) children, 8 of whom were RDT-negative and one was RDT-positive but slide-negative. Excluding 3 children with *S. typhi*, all of the children with bacteraemia were ≤12 months of age and had sensitivity to locally available antibiotics. Compared to double-read research slide results RDTs had a sensitivity of 97.8% (95%CI 96.9-98.7) and specificity of 96.3% (95%CI 96.3-98.4). Use of RDT's to direct the use of anti-malarial drugs in young children did not result in any missed diagnoses of malaria although new infections soon after a consultation with a negative RDT result may undermine confidence in results. Invasive bacterial disease is uncommon in children with non-severe illness and most cases occurred in infants with a current fever.

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REDUCTIONS IN ARTEMISININ-BASED COMBINATION THERAPY CONSUMPTION FOLLOWING THE NATIONWIDE SCALE UP OF ROUTINE MALARIA RAPID DIAGNOSTIC TESTING IN ZAMBIA

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Malaria remains one of the primary reported public health problems in Zambia. To strengthen malaria diagnostic accuracy and thereby reduce the cost associated with treating misdiagnosed individuals with ACTs, the Zambian national malaria control program introduced rapid diagnostic tests (RDTs) for *Plasmodium falciparum*. Zambian policy now recommends that the use of parasite-based diagnosis for every suspected malaria case regardless of age group. However, parasite-based diagnosis is of limited use unless clinicians maintain high adherence to results in subsequent management. A review of facility records was conducted for the period of 2004-2009 in three districts of Zambia; a total of 25 facilities had usable records and were included in the analysis. Median rollout of RDTs at the individual facility level occurred in the first month of 2007. Descriptive analysis showed substantial reductions in the use of ACTs as RDT availability was scaled up. The average proportion of out-patient department attendees treated with an ACT was significantly lower after initiation of RDT testing compared to the pre-RDT period (4.8% vs. 12.9%, p=0.001). By 2009, the number of ACT treatments provided essentially matched the number of RDT positive tests. Multivariate modeling using negative binomial and Poisson regression is being conducted to control for climate variability, vector control coverage, stock-outs of drugs and diagnostics and other potential confounders. Contrary to observations elsewhere in which impact on drug management has been poor, the implementation of RDT-based diagnosis in Zambia appears to be reducing and rationalizing the usage of ACTs even after controlling for a general downward trend in the malaria burden, and may thereby reduce ACT procurement costs while providing more accurate data on disease trends.

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PARASITE-BASED DIAGNOSIS OF MALARIA AMONG SYMPTOMATIC PREGNANT WOMEN IN A TERTIARY HOSPITAL IN AN ENDEMIC AREA

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Because malaria during pregnancy is often asymptomatic in malaria endemic areas, a high index of suspicion is maintained and pregnant women are placed on IPT and also treated presumptively at the earliest suspicion. However, the WHO now recommends that malaria diagnosis be parasite-based as much as possible. We evaluated the prevalence of malaria parasitemia among pregnant women suspected of having malaria by microscopy and Paracheck™ (a histidine-rich protein-2 based malaria rapid diagnostic test, Orchid Biomedical Systems, Goa India) in the antenatal and emergency obstetrics care setting of the University College Hospital in Ibadan, southwest Nigeria between October 2009 and January 2011. The mean age was 30.8years ± 4.7. The prevalence of malaria parasitemia was 22.8% (170/746) and 24.5% (151/617) by microscopy and Paracheck™ respectively. The geometric mean parasite density was 2,091 (range 40-156,975/µL). HIV positivity rate was 7.8% (58/746) while 32.6% (243/746) of enrollees had received at least one dose of IPT-SP by the time of enrollment. 7.5% (56/688) had a temperature ≥ 37.5^{0}C. Women with a Temperature >37.4˚C were significantly more likely to have malaria parasitemia [p<0.0001]. Fever or a history of fever, headache, vomiting, chills and rigors were significantly positively correlated with malaria parasitemia. Over one third (264/746; 35.3%) admitted to haven had a previous attack of malaria in the index pregnancy before enrollment. 44.5% (109/245) of the cases were diagnosed presumptively while the remainder had microscopic diagnosis. ACTs [87; 33.6%], amodiaquine [84; 32.4%], chloroquine [27/259; 10.4%], sulfadoxine-pyrimethamine [17; 6.5%] and artesunate [11; 4.2%] were the most often mentioned antimalarial drugs used. There was no correlation between the presence of malaria parasite and parity or IPT use. The overall sensitivity and specificity of Paracheck were 69.9% and 88.2% respectively while at parasite densities ≥200/µL were 84.8% and 88.7% respectively. Positive predictive value was 66.9% while the negative predictive values for the two cut off parasite densities were 91.1% and 96.3% respectively. In conclusion, presumptive diagnosis of malaria in pregnancy will lead to over treatment. Parasite-based diagnosis of malaria during pregnancy is recommended.

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DEVELOPMENT OF A RAPID DIAGNOSTIC TEST (RDT) FOR SIMULTANEOUS DETECTION OF G6PD DEFICIENCY AND MALARIA INFECTION

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G6PD deficiency is the most common human enzyme deficiency affecting 400 million people and highly prevalent in malaria endemic areas. This deficiency appears to provide some protection from malaria infection, but it can also cause hemolysis after administration of some malarial drugs, especially primaquine. There is an urgent need of rapid diagnostic test (RDT) suitable for the field to screen glucose 6 phosphate dehydrogenase (G6PD) deficiency before treatment with malarial drugs. We have developed a rapid test in dry format assay for the qualitative detection of G6PD enzyme activity. The G6PD RDT test result using clinical samples correlated with the quantitative test results and detected around ≤4 IU/Hg (normal 12± 2.09 at 37 ˚C) as deficient. The cutoff will be adjusted

depending on the tolerance of red blood cells against primaquine in G6PD deficient patient. Accelerated stability studies showed that test strips were stable for 2 months at 45°C and 10 days at 60˚C. This G6PD test RDT can be combined with a malaria RDT which is capable of detectings less than 30 parasites per µl of blood and is stable for 2 years at 40˚C as a dual test. This dual test kit diagnoses malaria infection and G6PD deficiency at the same time using small amount of blood (less than 10 µl). The test is rapid (<10 min), simple to use, inexpensive, portable, and has no special storage requirement which is critical element for field use.

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DRIED *PLASMODIUM FALCIPARUM* POSITIVE BLOOD AS QUALITY CONTROL SAMPLES FOR FIELD MONITORING OF MALARIA RDT PERFORMANCE

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Malaria rapid diagnostic tests (RDTs) are critical to the WHO recommendation for parasitologic confirmation of all suspected malaria cases. RDT performance is compromised by the high temperature conditions typical of malaria endemic regions. Despite this limitation, methods to monitor RDT performance in the field after exposure to such conditions are lacking. Positive control samples are unavailable and comparing RDTs to blood smears is not ideal since RDTs detect parasite antigens and microscopy detects parasites. Furthermore, accurate slide reading requires expertise unavailable in most health facilities. Currently, no reliable quality control (QC) method exists for RDTs in the field. We determined the suitability of dried parasite positive blood as QC samples for malaria RDTs using 3 *Plasmodium falciparum* culture strains at 200 and 2000 parasites/µl (p/µl) on 10 high-performing (WHO/FIND Evaluation) RDT brands. After baseline testing, 50µl aliquots of parasite positive blood were air-dried overnight, stored at 35˚C, 25˚C or 4˚C for 1, 4 and 12 wks and then tested on the same RDTs after rehydration with PBS-tween. All dried blood at 2000p/µl retained reactivity (100% sensitivity) on all 10 RDT brands at all temperatures and times points for HRP2. At 2000p/µl, sensitivity on the pLDH (Pan) bands for 2 Combo tests was reduced to 80-89%. Dry blood at 200p/µl for all storage temperatures and time points were detected at 100% sensitivity on 6 of 10 RDTs for HRP2. The sensitivity on 2 of the 4 remaining RDTs was 100% up to 4 weeks of storage at all temperatures, dropping to 87.5% at wk 12 for wk 12 samples stored at 35˚C. Sensitivity of detection on pLDH bands was low (29%, range 0-100%) at 200p/µl; partly attributable to weak baseline reactivity, and thus RDT brand. In the absence of positive control antigens, well-characterized parasite positive dried blood can be successfully used as a simple tool for monitoring RDT, especially HRP2 test performance in the field. The sample used should be well characterized for its baseline reactivity on the RDT it is intended to monitor.

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GOING BELOW THE TIP OF THE ICEBERG: DOES MOLECULAR DETECTION OF MALARIA CHANGE OUR UNDERSTANDING OF EPIDEMIOLOGY AND CONTROL?

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Molecular detection of *Plasmodium falciparum* malaria by PCR-based techniques has revealed low density infections in individuals who would previously have been classified as uninfected by standard microscopy, fundamentally changing estimates of malaria infection prevalence. However, the relevance of submicroscopic cases to control programmes remains unclear. Here we develop a quantitative framework for describing the distribution of low density infections and discuss malaria epidemiology and control in light of these data. We analysed 114 population surveys in which *P. falciparum* prevalence was measured by both microscopy and PCR, allowing for measurement error with Bayesian methods. In line with previous results, we find a significant increase in the sensitivity of microscopy as the underlying transmission intensity increases, from 60% when PCR prevalence is over 80%. We explore a number of hypotheses which could generate this observed relationship between microscopy and PCR prevalence using mathematical models, and find that it can be captured by variation across endemicities in a combination of the following (1) average age of malaria infections, (2) rates of partially successful treatment, (3) PCR contamination and (4) genetic diversity of parasites. We find that PCR prevalence can be estimated relatively accurately from microscopy prevalence with the best-fitting model giving a 91% correlation between predictions and data. Combining our analysis with two available estimates of the infectiousness of submicroscopic infections to mosquitoes, our results suggest that their contribution to the infectious reservoir may be important mainly in low transmission settings. Where slide-prevalence is 50% of mosquito infections versus <10% where slide-prevalence is >60%. Therefore PCR detection is likely to be worthwhile in areas with low transmission, for example during active case detection programmes, but of less concern to control agencies in more highly endemic areas.

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WITHIN HOST AND WITHIN COMMUNITY POPULATION DIVERSITY OF *PLASMODIUM FALCIPARUM CSP* T CELL EPITOPES BY MASSIVELY PARALLEL PYROSEQUENCING IN LILONGWE, MALAWI

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Plasmodium falciparum circumsporozoite surface protein (*csp*) is the current leading candidate for a malaria vaccine with the RTS,S vaccine in a phase III clinical trial. Immunity appears to be mediated both through antibody responses and T cell responses. The C-terminal region of *csp* contains two T cell epitopes (Th2 and Th3) which are highly polymorphic. Genetic diversity of the parasite may in the long run affect the ability of any vaccine to prevent disease. Parasite genetic diversity within individuals and within communities is not adequately described by traditional genotyping methods because they fail to capture all variants. To better describe the diversity of the *csp* T cell epitopes in Lilongwe, Malawi, we employed massively-parallel pyrosequencing (Roche 454 System) to sequence a 319bp amplicon of *csp* containing the Th2 and Th3 epitopes from 100 participants with uncomplicated malaria. Over 470,000 sequences aligned to the region of interest, of which about 360,000 were used to generate haplotypes and frequencies. We detected over 80 unique genetic haplotypes in the population. The average multiplicity of infection was 3.4 variants (range: 1-16). Using population genetic analysis methods, we estimated the total number of genetic haplotypes within the community and compared the diversity of parasite populations between adults and kids. The combination of ultra-deep sequencing and population genetic analysis provides tools to study and exploit the genomic diversity of *P. falciparum* within individual infections and within communities.

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A NOVEL ASSAY FOR IDENTIFYING GAMETOCYTOCIDAL COMPOUNDS AGAINST *PLASMODIUM FALCIPARUM*

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The apicomplexan parasite *Plasmodium falciparum* that causes malaria, still manages to kill up to 1 million people a year. Most of these deaths

occur in sub-Saharan African, where cheap and effective drugs are not always available or used efficaciously. In order to eradicate malaria it will be necessary to find drugs that can effectively kill all of the erythrocytic stages of the parasite, including gametocytes. Gametocytes are the only stage that leads to transmission of the disease from the human host to the mosquito vector. Gametocytes are difficult to study as unlike the asexual stages of the parasite they do not replicate in culture, as they are terminally differentiated. In order to find effective anti-transmission blocking drugs for malaria a method was required to produce sufficient gametocytes to be able to conduct drug assays. Gametocytes do not synthesise hypoxanthine, so a novel method of determining parasitic growth inhibition was also needed. Here we report on a novel assay method that enables drugs and compounds to be tested and novel gametocytocidal compounds to be identified. A method has been devised that allows large numbers of gametocytes to be produced and used in an assay that measures ATP production. The assay measures the number of live gametocytes after drug treatment and hence the gametocytocidal activity of compounds. The assay was tested against a small blinded library of compounds to provide a proof of concept. The ATP assay was able to identify a small number of compounds with previously known gametocytocidal activity and provide IC_{50}s against late stage gametocytes, for these compounds. The assay is now being optimised for high throughput screening to provide the first HTS assay for gametocytocidal compounds. This research has provided the first step in being able to identify drugs that will block the transmission of malaria and aid in the eventual eradication of this disease.

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VERY LONG LASTING EFFECT OF INTRA-MUSCULAR DECOQUINATE IN THE *PLASMODIUM CYNOMOLGI BASTIANELLII* (B STRAIN) RELAPSING MALARIA MONKEY MODEL

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Global eradication of malaria will require simple, safe treatment regimens to eliminate the persistent liver stage associated with *Plasmodium vivax* and *P. ovale*. Currently the only FDA approved treatment for this indication is primaquine. Unfortunately, the 8-aminoquinoline (8AQ) class is associated with hemolytic anemia in persons deficient in glucose-6-phosphate dehydrogenase (G6PD) necessitating the need for new drugs that are effective against hypnozoites without the G6PD liability. A number of non-8AQ compounds have been reported to possess anti-hypnozoite activity against sporozoite-induced *P. cynomolgi B* infections in Rhesus monkeys, including the quinoline esters WR197236 and WR194905. However, radical cures eliminating dormant hypnozoites were achieved only when the compounds were injected intramuscularly (IM). Decoquinate (DQ), a coccidiostat approved by the FDA for use in veterinary medicine, is structurally similar to WR194905 and if effective against hypnozoites could be pursued as a non-8AQ option. When co-administered with chloroquine (CQ), DQ cleared relapsing *P. cynomolgi* parasites from two infected Rhesus monkeys for over 100 days, a positive test for radical cure as established in the model (DQ: 15 mg/kg IM daily for 7 days, CQ: 10 mg base/kg oral daily for 7 days). Upon extended monitoring, however, relapsing parasitemia was observed well over one year after the initial clearance (days 509 and 511 after the last day of drug dosed). Two monkeys administered double the aforementioned dose of DQ (30 mg/kg IM once daily for 7 days) remained clear of parasitemia at this time. When DQ was co-administered orally (either 20 or 40 mg/kg BID for 14 days) with CQ, only one monkey in the high dose group showed any delay in parasitemia when compared to the purely anti-erythrocytic CQ controls. These results strongly suggest that the absence of relapse in the higher IM DQ dose is not a true radical cure, *i.e.* - elimination of all stages of parasites, but rather parasite suppression by long-lasting circulating drug levels from an IM depot effect.

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ESTABLISHMENT OF A RHESUS MONKEY MODEL TO PREDICT G6PD DEFICIENCY-RELATED HEMOLYTIC POTENTIAL FOR ANTIMALARIAL DRUG DEVELOPMENT

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Primaquine (PQ), an 8-aminoquinoline (8-AQ), is used for radical cure of *Plasmodium vivax* and to kill stage V *P. falciparum* gametocytes. However, this drug often triggers hemolytic anemia in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency. G6PD and glutathione (GSH) are known to protect red blood cells (RBCs) from oxidative damage leading to hemolysis. A suitable animal model is needed to screen new prospective nonhemolytic compounds. In this project, we sought to establish a nonhuman primate (NHP) model to closely mimic the hemolysis caused by PQ treatment in the G6PD deficient subjects. We hypothesized that GSH depletion of rhesus monkey RBCs (rmRBCs) *in vitro* and infusion of the GSH-depleted RBCs back into the animals could result in accelerated clearance of rmRBCs similar to hemolysis associated with PQ in human G6PD subjects. Blood was drawn from monkeys and subsequently treated with diethyl maleate and buthionine sulfoximine *in vitro* to deplete GSH to a residual level of 5-15 % and were labeled with a green fluorescent dye at the same time. The cells were then infused back to the original donors. PQ or other antimalarial drugs, i.e., tafenoquine (TQ), chloroquine (CQ), or mefloquine (MQ), were orally administered once a day immediately after the infusion. The blood samples were collected daily to monitor the fate of the RBCs by flow cytometry. PQ, either at 4 or 1.78 mg/kg, significantly accelerated clearance of GSH-depleted RBCs in a time-dependent manner and high dose TQ (6 mg/kg) also showed a significant acceleration of rmRBC clearance in comparison to control animals. In contrast, treatment with nonhemolytic drugs, CQ (25 mg/kg) and MQ (25 mg/kg), did not result in any accelerated rmRBC clearance. These findings clearly support our hypothesis that the artificially GSH-depleted rmRBCs mimic hemolysis seen in G6PD deficient patients treated with PQ. A NHP model is now available for antimalarial drug development and for identification of combinations to improve the therapeutic index of existing drugs.

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ENANTIOSELECTIVE METABOLISM AND PHARMACOKINETICS OF PRIMAQUINE IN PRIMARY HUMAN HEPATOCYTES, MICE AND NORMAL HUMAN VOLUNTEERS

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Primaquine (PQ) is clinically used in a racemic form. Earlier studies have indicated differential therapeutic profiles of PQ enantiomers. Enantioselective pharmacokinetic (PK) and pharmacologic characteristics may contribute to this property. Carboxy PQ (cPQ) has been demonstrated as a major plasma metabolite in animals as well as humans. Rate of

formation of cPQ could be a primary determinant of the pharmacologic/toxicological responses to this drug. Studies were undertaken to investigate enantioselective metabolism and PK of PQ *in vivo* in mice, normal human volunteers and *in vitro* in primary human hepatocytes. A chiral LC-MSD-TOF method for simultaneous analysis of enantiomers of PQ and cPQ was employed. In a PK study in mice administered a single 30 mg/kg dose of racemic PQ, (+) and (-)PQ exhibited similar plasma PK profile. However plasma (-)PQ level declined more rapidly compared to (+) PQ. A pronounced difference was noted in the plasma PK profile of cPQ enantiomers. The Cmax for (-)cPQ was >10 fold higher than (+)cPQ. A study conducted in normal human volunteers, after a single oral dose of 45 mg racemic PQ, confirmed differential PK profiles of PQ enantiomers. The plasma cPQ peaked at about 8 hours after drug administration and remained elevated for 24 hours; all of the plasma cPQ was due to the (-)cPQ, and (+)cPQ levels were under the detection limits, while plasma levels of both (+) and (-)PQ were low and variable throughout the study period. Primary human hepatocytes also differentially metabolized the PQ enantiomers. (-)PQ was more rapidly metabolized to (-)cPQ as compared to (+)PQ. Inhibitor studies suggested a major role of monoamine oxidase-A for formation of cPQ. These studies confirm that the two enantiomers of PQ have differential metabolic and PK profiles, which suggests they likely will have different efficacy and toxicity profiles. The studies strongly support further clinical evaluation to find if one enantiomer will afford a better therapeutic value over another.

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EFFECT OF CYP 3A4 INHIBITORS ON THE HEMATOLOGICAL TOXICITY AND PHARMACOKINETICS OF AN 8-AMINOQUINOLINE DEVELOPMENTAL CANDIDATE, NPC1161B

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8-Aminoquinoline antimalarial drugs (8-AQs) have broad utility and excellent efficacy, but also have limitations due to hematological toxicity in subjects with glucose-6-phosphate dehydrogenase deficiency (G6PDd). We previously showed that NPC1161B (NPCB), the (-) (*R*) enantiomer of (±) (*RS*)-8-[(4-amino-1-methylbutyl)amino]-6-methoxy-4-methyl-5-[3,4-dichlorophenoxy] quinoline succinate, proved extremely efficacious in animal models of malaria and leishmania. *In vitro* studies suggested that multiple human cytochrome P450 (CYP) isoforms mediate the generation of methemoglobin (mtHb) in the presence of the (+) enantiomer; however, for the (-) enantiomer (NPCB), CYP3A4 was the major contributor. The present studies assessed whether CYP3A4 inhibitors could block the hematological responses of NPCB in dogs. In one study, ketoconazole (KTZ) was administered p.o. (200 mg/kg), at 1 hr before and 11 and 23 hr after, a single oral dose of NPCB at 15 mg/kg. NPC caused a sustained rise in mtHb, from baseline of 1% to peak at 10% on d 6, with a slow decline, remaining elevated at 8% on d 12. KTZ administration inhibited the rise in mtHb (3% at d 6 and 4% at d 12). KTZ increased plasma levels of NPCB by about 50%. In the next study, NPCB was given at 15 mg/kg/d p.o. on days 1-4, and ritonavir (RTN) was given at 10 mg/kg p.o. twice a day on days 1-8. A marked increase in mtHb (peak at around 20% on day 8-10) was observed in the absence of RTN, with an increase in reticulocytes (RET) and nucleated red blood cells (nRBC). RTN completely prevented the rise in mtHb, RET and nRBC, suggesting that the hematological changes are indeed dependent on a CYP3A4-mediated pathway. However, unexpectedly, it was observed that RTN reduced peak plasma levels and AUC for NPCB by 55%, likely due to decreased intestinal absorption of NPCB in the presence of RTN. In conclusion, two CYP3A4 inhibitors reduced the metHb formation and erythropoiesis after high doses of NPCB. However, their effects on pharmacokinetics of the drug were divergent, and other inhibitors should be evaluated.

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OZ439 IN MALARIA PATIENTS, PRELIMINARY RESULTS FROM A PROOF OF CONCEPT STUDY IN ADULTS WITH *PLASMODIUM FALCIPARUM* OR *P. VIVAX* INFECTION

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OZ439 is a synthetic endoperoxide (1,2,4-trioxolane) which shows great promise in preclinical studies as part of a single dose cure for uncomplicated malaria. *In vitro*, it kills the parasite with a similar parasite reduction rate as artemisinin, and in phase I studies, a plasma concentration above the IC_{90} can be maintained for >72 hours. We have studied OZ439 in an open-label, phase IIa trial in 60 patients (two groups of 10 patients per dose cohort) with either uncomplicated *Plasmodium vivax* or *P. falciparum* malaria mono-infection (ClinicalTrials.gov: NCT01213966). After enrolment, patients received a single dose of OZ439. Patients were monitored for parasite load and pharmacokinetics every 6 hours, and standard of care treatment was administered between 36 and 72h. The primary efficacy endpoint was parasite reduction rate (PRR). OZ439 was well tolerated. Contrary to the results we obtained with a previous endoperoxide, OZ277 (Rbx11160), the plasma exposure of OZ439 was similar to that seen in volunteers. OZ439 was fast acting, with a Parasite Reduction Rate comparable to the PRR of three day artesunate treatment in hyperparasitemic patients. These data support a key role for OZ439 as one component of a new combination single dose cure for malaria.

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EFFICACY OF A TWO- VERSUS THREE-DAY COURSE OF DIHYDROARTEMISININ-PIPERAQUINE IN NORTHERN CAMBODIA: RESULTS FROM AN OPEN-LABEL, RANDOMIZED CLINICAL TRIAL

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Declining efficacy of artesunate-mefloquine has prompted a recent change to dihydroartemisinin-piperaquine (DP) as the first line therapy against all malaria infections in Cambodia. Although 3 days of DP

therapy is recommended in the civilian sector, a 2-day regimen of DP is currently used by the Cambodian military to improve compliance. As part of an active observational malaria epidemiology cohort study in Oddar Meancheay Province, an area of high transmission in northern Cambodia, 200 healthy volunteers were enrolled and followed weekly for up to 4 months. All subjects developing uncomplicated malaria were randomized to receive directly observed therapy with 320/2880 mg of DHA-piperaquine given over 2 or 3 days (n = 40 per arm). Subjects were followed weekly for a minimum of 42 days and assessed for treatment efficacy, safety and tolerability. The trial was powered (80%) to detect an expected 25% higher recurrence rate in the 2-day group compared to 3 days. From September 2010 to February 2011, 80 malaria patients were randomized to DP, 16 (20%) with *P. falciparum*, 61 (76%) with *P. vivax* and 3 (4%) with mixed infection. PCR-uncorrected per protocol 42-day efficacy rates against all malaria species combined were not statistically significantly different between treatment groups: 89% (95% CI 76-96%) for 2 days and 92% (95% CI 80-97%) for 3 days. Intention to treat efficacy rates were also not significantly different: 83% (95% CI 68-91%) for 2 days and 88% (95% CI 74-95%) for 3 days. Median parasite clearance times were 11.1 hours for *Plasmodium vivax*, but 72.5 hours for *P. falciparum*; there were no significant differences between treatment groups. DP was safe and well tolerated without significant treatment-related adverse events. PCR uncorrected all-malaria efficacy was not significantly different between 2 and 3 days of DP in this population on the Thai-Cambodian border. However, 42-day cure rates appear to be lower than previously reported. Given the proximity of this study site to areas of known multi-drug resistance this finding is concerning.

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CHROMOSOMAL INTEGRATION OF TRANSGENES AND DERIVATION OF A STABLE TRANSGENIC LINE IN THE PARASITIC NEMATODE *STRONGYLOIDES RATTI*

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Parasitic nematode infections adversely affect over one billion people. Genetic transformation is a potential tool for analyzing gene function to identify new drug and vaccine targets in these worms. We have developed a robust system for transgenesis in *Strongyloides* spp. using gonadal microinjection for gene transfer. Using this system, transgenes are expressed in promoter-regulated fashion in the F1 but are silenced in subsequent generations, presumably because of their location in repetitive episomal arrays. To counteract this silencing, we explored transposon-mediated chromosomal integration of transgenes in *S. ratti*. To this end, we constructed a donor vector encoding green fluorescent protein (GFP) under the control of the *Ss-act*-2 promoter with flanking inverted tandem repeats specific for the *piggyBac* transposon. Free-living *Strongyloides ratti* females were transformed with this donor vector and a helper plasmid encoding the *piggyBac* transposase. The transgene was detected in the F1 and later generations by PCR, and 15.8% of F1 larvae were GFP-positive. We inoculated a rat with 34 F1 GFP-positive infective larvae (L3i), and 0.48% of 6014 F2 individuals resulting from this host passage expressed GFP. We cultured GFP-positive F2 individuals to produce GFP-positive F3 L3i for additional rounds of host and culture passage. GFP expression frequencies in subsequent generations were 74.24% in F3, 98.99% in F4, 82.39% in F5 and 100% in F6. The resulting transgenic line now has uniform GFP expression among all progeny. Chromosomal integration of the reporter transgene in *S. ratti* was confirmed by Splinkerette PCR, which revealed the transgene flanked by *S. ratti* genomic sequences corresponding to at least three discrete integration sites. BLAST searches of flanking sequences against the *S. ratti* genome revealed integrations in three contigs: 75336 (position 3211), 74996 (position 155901) and 74278 (position 172601). This result provides the basis for two powerful functional genomic tools in *S. ratti*: hertiable transgenesis and insertional mutagenesis.

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NEOMYCIN SELECTION OF TRANSGENIC *SCHISTOSOMA MANSONI*

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Draft genome sequences for *Schistosoma mansoni* and *S. japonicum* were reported recently, a landmark event that ushered in the post-genomic era for schistosomiasis. Analysis of target genes to underpin new interventions for schistosomiasis requires functional genomics approaches such transgenesis that will validate essential genes to be targeted with drugs or vaccines. We have adapted murine leukemia retrovirus (MLV) vectors -widely used in human gene therapy research- to transduce schistosomes, leading to integration of reporter transgenes into the parasite genome. Drug selection of transgenic schistosomes would be highly desirable in order to provide a means to enrich for populations of transgenic worms in virion-exposed parasites. Given that *neoR* (the gene encoding resistance to neomycin/G418) driven by the MLV's 5'-LTR as promoter is actively expressed in schistosome tissues, and that G418 is lethal under the conditions tested here, we investigated whether MLV transduced schistosomules could be rescued on G418. First, a dose-response kill curve and lethal G418 concentrations were established. Second, one day old schistosomules were infected with MLV at two concentrations of virions, 1X and 3X. Transduced worms were cultured with or without G418 and by day 10, aliquots of schistosomules from the groups were stained for viability with Trypan blue and enumerated. No significant differences were observed among the group of parasites without G418. However, significant differences were found among schistosomules cultured with G418 where more schistosomes survived when transduced with virions (3x) in comparison to controls (p=0.0039). Remarkably, *neoR* expression levels in the group subjected to G418 selection was higher than in worms treated with the same titer of virus but cultured without G418. This likely reflects enrichment of transgenic schistosomules within the population of transduced parasites subjected to G418 pressure. This appears to be the first report of antibiotic selection of transgenic schistosomes or indeed of any transgenic helminth parasite species.

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THE COMPLETE *WOLBACHIA* GENOME AND TRANSCRIPTOME FROM *ONCHOCERCA OCHENGI* INDICATES A DIFFERENT WORM-SYMBIONT RELATIONSHIP TO THAT OF *BRUGIA MALAYI*

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The filarial nematode *Onchocerca ochengi*, a parasite of cattle, is recognised as the closest relative of *O. volvulus*, the aetiological agent of human onchocerciasis. In common with the filariae that cause lymphatic filariasis (including *Brugia malayi*), *O. ochengi* and *O. volvulus* contain *Wolbachia* endobacteria in the hypodermal cords of both sexes and in the reproductive tract of female worms. *Wolbachia*, which are much more prevalent in arthropods than in nematodes, are divided into approximately 10 supergroups. Four complete *Wolbachia* genomes have been published to date: two in supergroup A (from *Drosophila* spp. hosts), one in supergroup B (from a mosquito host), and one in supergroup D (strain *w*Bm from *B. malayi*). Here, we report the first complete genome of a supergroup C *Wolbachia* (strain *w*Oo from *O. ochengi*), alongside complete endobacterial transcriptomes obtained by deep sequencing of cDNA from both hypodermal cord and female reproductive tract tissues. At 0.96 Mb, the *w*Oo genome is the smallest thus far characterised for any *Wolbachia* and is 11% smaller than that of *w*Bm. In contrast to *w*Bm,

the *w*Oo genome contains very few insertion sequences and fewer intact ankyrin-repeat containing genes. Key metabolic pathways for heme and riboflavin, which have been hypothesised to form the basis of a nutritional mutualism between *w*Bm and its worm host, show evidence of pseudogenization in *w*Oo and transcription across both pathways is low, irrespective of anatomical location in the worm. The *w*Oo transcriptome is dominated by chaperonin and chaperone proteins, translation machinery and enzymes involved in nucleotide metabolism. Approximately 100 proteins encoded by abundant *w*Oo transcripts were detected in worm homogenates by proteomic methods. Three of these are uncharacterised membrane proteins, two of which do not have orthologues in *w*Bm. Taken together, these analyses indicate important dissimilarities between the genomes of *w*Bm and *w*Oo, and suggest that immuno-defensive (as opposed to nutritional) mutualism may be the major phenotypic role of *w*Oo in its worm host.

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FUNCTIONAL GENOMICS APPROACHES TO STUDYING *SCHISTOSOMA MANSONI* FEMALE WORM DEVELOPMENT

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Schistosomiasis affects more than 200 million people worldwide making it a major cause of morbidity and mortality globally. Although the genome sequence for *Schistosoma mansoni* has been determined, few functional genomics techniques have been developed for use in *S. mansoni,* limiting the usefulness of the sequence data. Using serial analysis of gene expression (SAGE) Williams et al (2007) elucidated transcriptional differences between life cycle phases. Using the SAGE data we have identified transcripts that are preferentially expressed in reproductively mature females. Using bioinformatics, we have linked these SAGE tags to unique protein sequences; however, many of these female-specific proteins do not have a predicted function. To functionally characterize these transcripts we have developed a whole mount *in situ* hybridization (WISH) method that can be used to identify the tissue-specific expression of transcripts in intact, adult *S. mansoni* worms (Cogswell et al 2011). To validate these protocols we determined the tissue-specific expression of *tetraspanin 2, phenol oxidase*, the secretory *Cu/Zn superoxide dismutase*, and an Argonaute family member. The localization of these transcripts by WISH correlates with prior studies performed using other methods, indicating that WISH will be a useful functional genomics tool. We have also identified a variety of cell-specific markers (Collins et al, 2011) to be used in conjunction with WISH to pinpoint the tissue-specificity of expression of female-specific transcripts providing more information about their function. Using WISH we have localized expression of four female-enriched transcripts specifically to the vitellaria/vitelline duct and three transcripts to the ovary, oviduct, and/or ootype. In order to determine the function of these female-enriched transcripts we have investigated the use of the phylogenetically related flatworm *Schmidtea mediterranea* as a model for schistosome biology. We have identified orthologs of many of the *S. mansoni* female-enriched genes in *S. mediterranea.* We have localized several of these orthologs in *S. mediterranea* using WISH and find that they localize to reproductive tissues in the same way that we observed in *S. mansoni.* Currently we are using established RNA interference techniques to silence orthologs in both worm species to examine what role they play in female development and reproductive biology.

1003

ANALYSIS OF TRANSCRIPTIONAL REGULATION OF TETRACYCLINE RESPONSIVE GENES IN *BRUGIA MALAYI*

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Most filarial parasites, including *Brugia malayi* harbor an endosymbiotic bacterium of the genus *Wolbachia*. While the physiological role of the endosymbiont remains unclear, it's elimination by doxycycline or tetracycline treatment results in sterilization of the adult parasite. Previous studies have demonstrated that the mRNA pools of certain nuclearly encoded genes are increased in parasites exposed to tetracycline, implicating these as potentially important players in the *B. malayi - Wolbachia* interaction process. It is possible to hypothesize that the increase in the stable mRNA levels of these genes results from up-regulation of transcription and that this involves cis acting regulatory sequences present in the gene's promoters. To test this hypothesis, the responsiveness of three promoters derived from genes whose mRNAs were increased by tetracycline treatment were tested in a homologous *B. malayi* transfection system. Reporter gene activity driven from all three promoters was found to increase upon exposure to tetracycline, consistent suggesting that transcription was up-regulated in response to tetracycline. The element responsible for tetracycline responsiveness was mapped in one of these promoters, BmHSP70. Mutation of the stress response element in the BmHSP70 promoter did not result in any change in tetracycline responsiveness. However, mutation of a TATAA box-like motif present in the promoter resulted in loss of the tetracycline response. These studies provide evidence supporting the hypothesis that changes in mRNA levels in response to tetracycline treatment are regulated at the transcriptional level and that this is accomplished through a novel use of an element normally employed as part of the core of many eucaryotic promoters.

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EVIDENCE OF EFFICIENT TRANSOVARIAL TRANSMISSION OF CULEX FLAVIVIRUS BY *CULEX PIPIENS* (*DIPTERA: CULICIDAE*)

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The purpose of this study was to determine the transovarial transmission (TOT) potential and tissue tropisms of Culex flavivirus (CxFV), an insect-specific flavivirus, in *Culex pipiens* (Linnaeus). Several hundred mosquito egg rafts were collected in the field, transferred to the insectaries, reared to the fourth larval stage and identified using morphological characteristics. *Cx. pipiens* were reared to adults, allowed to oviposit in individual containers and tested for CxFV RNA by reverse transcription-polymerase chain reaction (RT-PCR) and nucleotide sequencing. Eighteen CxFV RNA-positive females were identified. Thirty F_1 adults from each positive female were individually tested by RT-PCR for CxFV RNA. Viral RNA was detected in 526 of 540 progeny and thus, the filial infection rate was 97.4%. Because all 18 females produced infected offspring, the TOT rate was 100%. These data suggest that efficient TOT of CxFV occurs in nature. To define the tissue tropisms of CxFV, different tissues (salivary glands, ovaries, testes, head, fat bodies and midguts) were removed from the remainder of the F_1 and tested by RT-PCR for CxFV RNA. Viral RNA was detected in all tissues. Additionally, uninfected laboratory-colonized *Cx. pipiens* were infected with CxFV by needle inoculation, and ovaries were collected at 4, 6, 8 and 12 days post-inoculation and tested for CxFV RNA by RT-PCR. Viral RNA was detected at all time points demonstrating that

CxFV reaches the ovaries as early as 4 days post-inoculation. Surprisingly, however, we were unable to demonstrate transovarial transmission despite the presence of viral RNA in the ovaries. Nevertheless, the experiments performed with field-infected *Cx. pipiens* demonstrate that TOT is an efficient mechanism by which CxFV is maintained in mosquitoes in nature.

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CHIKUNGUNYA VIRUS IN VERTEBRATES

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Chikungunya virus (CHIKV) is a mosquito-borne alphavirus (family *Togaviridae)* endemic to Africa and much of Southeast Asia that can cause severe debilitating fever and arthralgia in humans. The African strains of CHIKV are thought to have a sylvatic cycle maintained by rodents and forest mosquitoes with occasional outbreaks in human populations. In Asia, there is no known sylvatic reservoir and the virus is proposed to circulate exclusively in humans. Recent viral evolutionary changes have been linked to increased virulence and altered vector specificity, such that some recent outbreaks have been associated primarily with *Ae albopictus,* while historically *Ae aegypti* have been the predominant human vector. The purpose of this study was to investigate the potential of various domestic and wild vertebrates to become infected with and possibly contribute to the spread of CHIKV. We tested more than 30 species representing all four classes of terrestrial vertebrates: reptiles, amphibians, mammals and birds. Animals were inoculated subcutaneously with 10,000-100,000 plaque-forming units of either a South African strain of CHIKV or an isolate from the Comoros Islands 2005 outbreak. Blood samples were collected daily for at least six days to characterize viremia. None of the birds developed detectable viremia, and of the mammals tested, only lab rodents demonstrated viremia. However, several of the ectothermic species, including leopard frogs, garter snakes, Burmese pythons, and ball pythons became viremic and maintained virus at sufficiently high titers to potentially re-infect mosquitoes. No mortality or obvious signs of clinical illness were observed in any of these species. These experiments suggest that reptiles and amphibians may play a role in virus maintenance and spread during epidemic seasons. Additionally, it is possible that hibernating ectotherms could provide a mechanism for virus overwintering in climates where winter months don't support vector populations.

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CHIKUNGUNYA VIRUS EMERGENCE IS CONSTRAINED IN ASIA BY LINEAGE-SPECIFIC ADAPTIVE LANDSCAPES

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Adaptation of RNA viruses to a new host or vector species often results in emergence of new viral lineages. However, lineage-specific restrictions on the adaptive processes remain largely unexplored. Recently, a chikungunya virus (CHIKV) lineage of African origin emerged to cause major epidemics of severe, persistent, debilitating arthralgia in Africa and Asia. Surprisingly, this new lineage is actively replacing endemic strains in Southeast Asia that have been circulating there for 60 years. This replacement process is associated with adaptation of the invasive CHIKV strains to an atypical vector, the *Aedes albopictus* mosquito that is ubiquitously distributed in the region. Here we demonstrate that linage-specific epistatic interactions between substitutions at amino acid positions 226 and 98 of the E1 envelope glycoprotein, the latter of which likely resulted from a founder effect, have for 60 years restricted the ability of endemic Asian CHIKV strains to adapt to this new vector. This adaptive constraint appears to be allowing invasion of the unoccupied vector niche by *Ae. albopictus*-adapted African strains. These results underscore how different adaptive landscapes occupied by closely related viral genotypes can profoundly affect the outcome of viral evolution and disease emergence.

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IDENTIFICATION AND CHARACTERIZATION OF CHIKUNGUNYA VIRUS VARIANTS WITH INCREASED TRANSMISSION *IN VIVO*

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Chikungunya virus (CHIKV), an Alphavirus and member of the Togaviridae family, is a re-emerging and significant human pathogen. Like all other arboviruses, CHIKV is able to infect and face numerous selective pressures and bottlenecks in both vertebrate and invertebrates hosts, yet the molecular mechanisms involved in initiating, establishing, and maintaining these distinct infections are poorly understood. Genetic diversity and adaptive mutations have been shown essential for the evolution of CHIKV host tropism, suggesting this as a possible mechanism important in mediating viral infection. Thus, we hypothesized that genetic adaptations *in vivo* may play important roles in CHIKV transmission and pathogenesis as well. To address this hypothesis, we infected *Aedes aegypti*i mosquitoes with wildtype CHIKV and harvested infectious virus from insect midguts, legs, salivary glands, and saliva at various time points. We found evidence for genetic bottlenecks at the level of midgut and salivary glands that were overcome downstream of these events, and we subsequently sequenced viral genomes present in each fraction to determine the genetic changes that occurred over the course of infection. Interestingly, we identified a major sub-population of viruses containing two previously undescribed mutations in the E1 glycoprotein present only in the saliva samples. These mutations were individually introduced into the Chikungunya virus infectious clone, virus was produced, and both mosquitoes and mice were infected. We found both mutations to significantly increase transmission rates compared to the wildtype control, suggesting that these variants comprise a temporally or anatomically restricted sub-population important in completing the transmission cycle. Further *in vivo* and *in vitro* studies are in progress to identify the molecular mechanisms involved. These studies will not only increase our understanding of CHIKV biology but should also provide valuable insight into the mechanisms of arbovirus evolution and pathogenesis.

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PRECLINICAL DEVELOPMENT OF A LIVE-ATTENUATED CHIKUNGUNYA VACCINE

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Recently, Chikungunya virus (CHIKV), a mosquito-borne alphavirus, re-emerged in Africa and spread to islands in the Indian Ocean, the Indian subcontinent, SE Asia and Italy. Viremic travelers have also imported CHIKV to the Western hemisphere, which highlights the risk of CHIKV in naïve populations. In addition to the huge burden of arthralgic disease, which can persist for months or years, epidemiologic studies estimated case-fatality rates of ~0.1%, principally from neurologic disease in older patients. There are no licensed CHIK vaccines or effective therapies. CHIKV is endemic in many resource-poor countries, so an effective vaccine must be inexpensive to manufacture and induce rapid, long-lived immunity. To develop a live-attenuated CHIK vaccine (CHIKV-IRES), we inactivated the subgenomic promoter of CHIKV La Reunion and inserted a picornavirus internal ribosome entry site (IRES) that functions poorly in insect cells. This vaccine is highly attenuated yet immunogenic in mouse models, and

is incapable of replicating in mosquito cells. We have characterized the safety and toxicology of this vaccine strain in A129 and C57/Bl6 mouse models, and have demonstrated its attenuation as compared to the 181-/clone 25 CHIK vaccine. To support our preclinical efforts, we have characterized the genetic stability of CHIKV-IRES *in vitro*, developed quality control and release assays, and initiated GMP vaccine manufacture which will support the testing necessary to submit an IND application to begin clinical trials in humans. This innovative collaboration between academic and industrial partners will have immediate, dramatic impacts on human health in Asia and Africa where CHIKV causes both severe health effects and economic hardship. A safe and effective CHIKV vaccine could, also greatly reduce the risk of CHIKV importation and endemic establishment in the Western Hemisphere during epidemics in Africa or Asia.

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SINGLE DOSE RVF-VRP IMMUNIZATION CONFERS COMPLETE PROTECTION FROM LETHAL RIFT VALLEY FEVER VIRUS INFECTION REVEALING THE IMPORTANCE OF VIRAL REPLICATION FOR ANTIVIRAL IMMUNITY

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Rift Valley fever virus (RVFV) is a mosquito-borne pathogen that poses a significant threat to human and livestock health throughout Africa and the Arabian Peninsula. Abortion storms in sheep and cattle are characteristic of RVF epizootics, and mortality of young animals can approach 100%. RVFV infection in humans is generally associated with a self-limiting febrile illness, but in a small percentage of cases (1-2%), it can progress to severe hepatitis, delayed-onset encephalitis, retinitis or a hemorrhagic syndrome with case fatality of 10 to 20%. In an effort to improve upon previously reported virus-like particle (VLP) vaccine constructs, we developed a system for producing high-titered (>$5.0x10^6$ FFU/ml) infectious non-spreading viral replicon particles (RVF-VRP). These constructs differ from authentic RVF virus in that RVF-VRP undergo only one round of infection; new particles cannot be produced in infected cells due to the absence of the glycoprotein-encoding viral M segment. Unlike standard VLP, RVF-VRP contain the full-length viral RdRp-encoding L segment and the nucleoprotein-encoding S segment, allowing *de novo* synthesis of all viral and/or marker proteins (i.e., GFP and luciferase). This active replication within infected cells suggests RVF-VRP should be more highly immunogenic than traditional VLP vaccine constructs. A single dose immunization with RVF-VRP ($1x10^5$ FFU SC) in C57BL/6 mice provided 100% protection from lethal RVFV challenge ($1x10^5$ PFU SC) at 28 days post-immunization. In contrast, immunization with non-replicating VLP controls resulted in lower survivorship after lethal virus challenge, indicating RdRp-mediated replication is important for the development of an effective immune response. Future studies will elucidate the mechanism by which viral replication in host cells enhances the immune response to protect against this significant health threat.

1010

HUMAN MONKEYPOX IN THE AFTERMATH OF SMALLPOX ERADICATION AND THE RISK OF SUSTAINED HUMAN-TO-HUMAN TRANSMISSION

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Since the eradication of smallpox three decades ago, human cases of zoonotic orthopoxvirus infections have risen worldwide. The lack of continued smallpox vaccination is likely a contributing factor because it decreases population immunity, but the 20-fold increased incidence of human monkeypox in the Democratic Republic of the Congo is particularly striking. By combining contact tracing data with scar surveys indicative of existing immunity to orthopoxvirus, we show that monkeypox virus in its present form is unlikely to establish persistent circulation in the rural populations currently affected. However, viral adaptation or introduction to crowded urban settings poses major risks. Focusing on the contribution of human to human transmission against a background of zoonotic spillover, we analyze surveillance data from 1980-84 and 2005-07 to show that the marked increase in human cases cannot be explained by reduced population immunity alone, and that increased spillover probably has contributed substantially. Further, we demonstrate the importance of quantifying surveillance errors including imperfect case detection, multiplicity of primary infections and false positive cases when estimating the effective reproductive number. Thus our analysis provides perspective for future surveillance and subsequent analysis of monkeypox and other emerging zoonoses. More generally, the rise of monkeypox serves as a warning that pathogen eradication can lead to unintended consequences with potential to partially offset public health gains.

1011

DEVELOPMENT OF A RECOMBINANT PROTEIN BASED CHEMICAL CONJUGATE VACCINE TO INTERRUPT MALARIA TRANSMISSION

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The goal of elimination and eradication of *Plasmodium falciparum* (*Pf*) will only be achieved with the development of an effective malaria vaccine. Vaccines to Interrupt Malaria Transmission (VIMT) are a class of malaria vaccines recently identified as a key element to achieve this goal. A VIMT may target both pre-erythrocytic and sexual stage parasites to produce a bifunctional vaccine. To this end, a vaccine is being developed that uses recombinantly produced well characterized, non-tagged near full-length Pf circumsporozoite protein and the 25 kDa sexual stage specific protein, Pfs25M, both independently conjugated to the vaccine carrier EPA (ExoProtein A, a non-toxic mutant of ExoToxin A from *Pseudomonas aeruginosa*). Pfs25H-EPA conjugates, manufactured under cGMP, induce a significant increase in antibody responses, over unconjugated antigen, which corresponds with enhanced transmission blocking activity. Conjugation of recombinant CSP also significantly enhances CS specific antibody responses and may broaden the breadth of the response against the amino- and carboxyl-terminal ends. Identification of a common platform for adjuvant formulation is being initiated. Additional parasite proteins are under pre-clinical development, including the scalable production of another transmission blocking vaccine candidate Pfs230, which induces optimum transmission blocking activity in the presence of human complement. Altogether, this platform enhances the potential to develop an effective bifunctional VIMT vaccine.

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IMMUNOGENICITY OF MIXED AND SINGLE ALLELE VACCINES OF *PLASMODIUM VIVAX* DUFFY BINDING PROTEIN REGION II

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The Duffy Binding protein of *Plasmodium vivax* is essential for host erythrocyte invasion. DBP region II (DBPII) contains critical residues for receptor recognition thereby making the molecule an attractive vaccine candidate against *P. vivax* blood stages. Immune responses to DBP have been shown to inhibit erythrocyte binding and invasion. Similar to other blood-stage antigens, allelic variation within the DBPII and associated strain specific immunity may be a major challenge for development of a broadly effective vaccine against *vivax* malaria. We hypothesized that immunization with a multiple allele vaccine will be more effective in producing a broadly reactive and inhibitory antibody response to diverse DBPII alleles than a single allele vaccine. In this study, we compared single PvDBPII allele immunizations (Sal1, 7.18, P) with a combination of the same alleles. Quantitative analysis by ELISA demonstrated that the immunogenicity of the multiple allele vaccine strategy generally performed better when tested for reactivity against heterologous variant DBPII alleles, with significantly higher antibody titers induced by some of the single allele immunizations. Qualitative analysis by *in vitro* erythrocyte-binding inhibition assays demonstrated that the multiple allele immunization strategy overall produces a broader binding-inhibitory antibody response, even to alleles not included in the vaccine. In either case, there was no correlation between antibody titer and functional inhibition. These data suggest that a multiple allele vaccine may enhance immunogenicity of a PvDBPII vaccine and requires further investigation to optimize.

1014

EVALUATION OF THE *PLASMODIUM FALCIPARUM* ERYTHROCYTE INVASION LIGAND PFRH4 AS A TARGET OF PROTECTIVE IMMUNITY AND VACCINE CANDIDATE

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Repeated exposure to *Plasmodium falciparum* in humans eventually results in protective immunity that prevents symptomatic malaria and high-density parasitemia. Antibodies to *P. falciparum* merozoite antigens are thought to play an important role, but their targets have been poorly defined, few merozoite antigens have been examined, and antibody effector mechanisms are not well understood. Additionally, there is limited knowledge to guide the selection of candidate antigens for vaccine development. The *P. falciparum* reticulocyte binding homologues (PfRh1, PfRh2, PfRh4, and PfRh5) play an important role in invasion of erythrocytes and are potential vaccine candidates. PfRh4 plays a key role in sialic acid-independent invasion of erythrocytes and binds to CR1. However, the importance of PfRh4 as a target of acquired immunity has not been established and its expression is known to vary between different isolates. In this study, we undertook a comprehensive analysis of the significance of PfRh4 as a target of human immunity and its potential as a vaccine candidate. Using recombinant PfRh4 proteins we assessed the acquisition of antibodies to PfRh4 among a longitudinal cohort of 200 children in Papua New Guinea. Total IgG and IgG subclasses were assessed and prospectively related to the risk of symptomatic malaria and parasitemia of different densities. Antibodies against the erythrocyte-binding region of PfRh4 were affinity-purified from human sera and were shown to be potent inhibitors of erythrocyte invasion. Sequence analysis of PfRh4 identified no significant polymorphism and thus no evidence for diversifying selection. Examining PfRh4 expression in clinical *P. falciparum* isolates derived from the cohort showed that PfRh4 was expressed among most isolates, indicating its relevance as an immune target. Our results provide important insight into the development of naturally acquired immunity and support a PfRh4 as a potential vaccine candidate.

1015

VACCINE DELIVERY PLATFORM IMPACTS INHIBITORY ANTIBODY CROSS-REACTIVITY OF $MSP1_{42}$-BASED VACCINE

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Plasmodium falciparum MSP1 is a leading erythrocytic-stage malaria vaccine candidate. The 195kDa protein is processed into several fragments, and has been implicated in the initial binding of erythrocytes by merozoites. Prior to erythrocyte invasion, the C-terminal fragment known as $MSP1_{42}$ undergoes secondary processing yielding a 33 and a 19kDa fragment ($MSP1_{19}$). Although results from seroepidemiological studies have suggested that antibodies to fragments of $MSP1_{42}$, particularly $MSP1_{19}$, may correlate with reduced clinical disease and/or parasite densities, no $MSP1_{42}$-based vaccine to date has yielded clinical efficacy.

Development of efficacious blood-stage vaccines is complicated by pre-existing immunity and heterogeneity of circulating parasites observed in the field, thus we have undertaken to evaluate several approaches for delivering $MSP1_{42}$-based vaccines in rabbits. Our previous studies have shown that immunizing rabbits with recombinant $MSP1_{42}$ using a potent adjuvant, Complete Freund's, resulted in high antibody titers that cross-react against heterologous strains. We evaluated two approaches for delivering $MSP1_{42}$; one expressing the antigen on the surface or in the periplasmic space of inactivated *Escherichia coli* designated GeMI-Vax, and a second, admixture of the antigen with Neisseria Outer Membrane vesicles (NOMs) in order to develop a more clinically suitable vaccine. Both of these platforms are self-adjuvanted and thus do not require additional immunostimulatory components. $MSP1_{42}$specific antibodies will be assessed for their ability to inhibit parasite invasion and growth using a pLDH GIA against homologous and heterologous parasite strains. In addition, antibody fine specificities will be evaluated using allele specific $MSP1_{42}$ ELISAs and fragment-specific particle-based Luminex. Results from these studies will guide the development of second generation $MSP1_{42}$-based malaria vaccines and may transcend the issues of allele-specific monovalent subunit vaccines.

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POTENT VACCINE PLATFORMS CAN PROVOKE CIRCUMSPOROZOITE (CS) PROTEIN MEDIATED IMMUNOSUPPRESSION, A PARADOX OVERCOME BY CS VACCINES EXPRESSING EAT-2

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Malaria greatly impacts the health and wellbeing of over half of the world's population. Promising malaria vaccine candidates have attempted to induce adaptive immune responses to Circumsporozoite (CS) protein. Despite the inclusion of potent adjuvants, these vaccines have limited protective efficacy. Conventional recombinant adenovirus (rAd) based vaccines expressing CS protein can also induce CS protein specific immune responses, but these are essentially equivalent to those generated after use of the CS protein subunit based vaccines. In this study we combined the use of rAds expressing CS protein along with rAds expressing novel innate immune response modulating proteins in an attempt to significantly improve the induction of CS protein specific cell mediated immune responses. BALB/cJ mice were co-vaccinated with rAd vectors expressing CS protein simultaneous with a rAd expressing either a TLR agonist (rEA) or the SLAM receptors adaptor protein (EAT-2). Paradoxically, expression of the TLR agonist uncovered a potent immunosuppressive activity inherent to expression of the CS protein, an activity that prevented the rAd vaccine from inducing CS specific adaptive immunity. Fortunately, use of the rAd vaccine expressing EAT-2 circumvented CS protein's immunosuppressive activity, and generated a fivefold increase in the number of CS protein responsive IFNγ secreting splenocytes, as well as increased the breadth of T cells responsive to peptides present in the CS protein. These improvements were positively correlated with the induction of a fourfold improvement in CS protein specific CTL functional activity *in vivo*. Our results emphasize the need for caution when incorporating CS protein into malaria vaccine platforms expressing or containing other immunostimulatory compounds, as the immunological outcomes may be unanticipated and/or counter-productive. However, expressing the SLAM receptors derived signaling adaptor EAT-2 at the same time of vaccination with CS protein can overcome these concerns, as well as significantly improve the induction of malaria antigen specific adaptive immune responses *in vivo*.

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BLOOD STAGE MEROZOITE SURFACE PROTEIN CONJUGATED TO NANOPARTICLES INDUCE POTENT PARASITE INHIBITORY ANTIBODIES

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We report the results of our proof-of-concept studies on the use of <15nm, water soluble, inorganic nanoparticles as a vaccine delivery system for a blood stage malaria vaccine. Accordingly, the recombinant malarial antigen, Merozoite Surface Protein 1 (rMSP1) of *Plasmodium falciparum* was used as the model vaccine. The rMSP1 was covalently conjugated to polymer coated quantum dot CdSe/ZnS nanoparticles (QDs) via surface carboxyl groups, forming rMSP1-QDs. Anti-MSP1 antibody responses induced by rMSP1-QDs were at least two orders of magnitude higher than those obtained with rMSP1 administered with the conventional adjuvants, Montanide ISA51 and CFA. Moreover, the immune responsiveness and the induction of parasite inhibitory antibodies were significantly more superior in mice injected with rMSP1-QDs. The rMSP1-QDs delivered via intra-peritoneal (i.p.), intra-muscular (i.m.), and subcutaneous (s.c.) routes were equally efficacious. The high level of immunogenicity using the rMSP1-QDs were achieved without further addition of other adjuvant components. Bone marrow derived dendritic cells were shown to efficiently uptake the nanoparticles which lead to their activation and expression/secretion of key cytokines. Suggesting that this may be a mode of action for the enhanced immunogenicity. This study provides promising results for the use of water soluble, inorganic nanoparticles (<15nm) as potent vehicles/platforms to enhance the immunogenicity of polypeptide antigens in adjuvant-free immunizations.

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NEXT-GENERATION POPULATION GENOMIC SEQUENCING TO IDENTIFY FUNCTIONAL MICROSATELLITE VARIATION IN *PLASMODIUM FALCIPARUM*

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Genome-wide variation studies in *Plasmodium falciparum* to date have been primarily focused on SNPs and large structural variations. Low complexity features like microsatellites are abundant in the *P. falciparum* genome (~488,000 loci); however, microsatellites have length-variation mutation rates that are orders of magnitude higher than the base substitution rate that creates SNPs. These two observations therefore suggest that microsatellites have the potential to be a significant source of functional coding and regulatory variation throughout the genome. Many studies have focused on microsatellite variation in targeted regions of the genome, but genome-wide analyses using short-read next generation sequencing data have been hindered by the high AT content (~80%) of the genome. We developed a tool to interrogate genome-wide variation in microsatellite regions of the *P. falciparum* genome using short read Illumina sequencing data, and validated its accuracy with a comparative analysis of Sanger generated sequence from the 3D7 and Dd2 strains. Polymorphic microsatellite calls based on Illumina data were validated through PCR amplification and Sanger sequencing of a subset of loci. Genotypes for ~75,000 microsatellite loci were generated from Illumina sequencing data for a sample of 25 Senegalese parasite isolates. A total of 17,940 of these loci were identified as polymorphic in at least one isolate, 8,556 of which were polymorphic in at least two isolates. Furthermore, 282 polymorphisms observed in at least two isolates fall within genic coding sequences and likely induce frame-shift mutations. Genes subject

to microsatellite-based frame-shifts are found throughout the *P. falciparum* genome and span a range of functions, including chromatin regulation and cellular metabolism. The impact of microsatellite polymorphisms on cis-regulation of gene expression is more difficult to assess, but is potentially significant given that 1648 genes have a polymorphic microsatellite within 500 bp upstream of their translational start site. The genome-wide prevalence and variability of microsatellites at the population level suggests they should be considered on par with SNPs as contributors to important functional polymorphism in the parasite.

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POPULATION GENOMIC SCAN FOR SIGNATURES OF BALANCING SELECTION AND NOVEL CANDIDATE TARGETS OF IMMUNITY IN *PLASMODIUM FALCIPARUM*

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The memory component of acquired immune responses causes frequency-dependent selection on pathogens, leading to distinctive patterns of polymorphism in genes encoding important target antigens. These are detectable by evaluating statistical signatures of balancing selection, using either frequency-based or polymorphism-versus-divergence indices. This has been well illustrated in analyses of malaria parasite antigens that are known candidate targets of naturally acquired immunity and more recently in new panels of genes expressed in merozoites. For a comprehensive screen of such signatures and to prospect for more targets of immunity among poorly known *Plasmodium falciparum* proteins, we purified parasites from >100 isolates in an endemic Gambian population and obtained high coverage genome sequence data by paired-end Illumina shotgun reads for almost all protein coding genes in 65 isolates. Excluding large sub-telomeric gene families (Var, rifin and stevor) and after masking repeat sequences, we obtained high quality data for >70% of the coding sequence in most genes, including 2853 that contained at least 3 single nucleotide polymorphisms and thus sufficient information for analysis. From analysis of summary indices generally useful for identifying outlier loci, we identified 241 gene loci (5% of genome) with positive signatures of balancing selection. Our results were concordant for a majority of genes previously studied by capillary re-sequencing in independent population samples and we further identified candidate loci with even more extreme evidence of balancing selection in a large number of merozoite specific genes, now prioritized for functional study and vaccine candidacy.

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THE TRANSCRIPTION FACTOR T-BET REGULATES PARASITEMIA AND PROMOTES PATHOGENESIS DURING *PLASMODIUM BERGHEI* ANKA MURINE MALARIA

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$CD4^+$ T cells are required for the pathogenesis of experimental cerebral malaria (ECM) during the induction phase of disease in mice. Using the *Plasmodium berghei* ANKA murine model of ECM and mice deficient for the transcription factor T-bet (the master regulator of Th1 cells) on the C57BL/6 background, we demonstrate that while Th1 $CD4^+$ T cells play a role in the regulation of parasite burden, they also promote the pathogenesis of ECM possibly by invoking a robust proinflammatory response. T-bet deficient mice had higher parasitemia than wildtype controls during the ECM phase of disease (17.7 ± 3.1% verses 10.9 ± 1.5%). In addition, while 100% (10/10) of wildtype mice developed ECM by day 9 post-infection, only 30% (3/10) of T-bet deficient mice succumbed to disease during the cerebral phase of infection ($p<0.0001$). In depth analysis of immune cells and cytokines is currently being performed to better understand how Th1 $CD4^+$ T cells mediate their protective as well as pathogenic functions during malaria infection.

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CHEMOKINE LEVELS DURING PREGNANCY MALARIA ARE ASSOCIATED WITH REDUCTION IN BIRTHWEIGHT

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In malaria endemic areas, first time mothers are highly susceptible to malaria infection. The main risks associated with pregnancy malaria (PM) are reduction in birthweight and maternal anemia. During normal pregnancy, the placenta displays a bias toward type 2 cytokines. However, pregnancy malaria shifts the balance from type 2 to type 1 cytokines. In our previous studies, type 1 cytokines like IFN-γ and TNF-α were associated with reduction in birthweight. Here, we examined placental levels of inflammatory chemokines and related their levels with pregnancy outcomes. CXCL9 and CXCL13 were significantly higher among malaria-infected pregnant women of all gravidities. However, high chemokine levels negatively correlated with birthweight among first time mothers only. CXCL9 is one of the chemokines induced by IFN-γ previously shown to increase during PM. The results presented here further expand our understanding of the mechanisms leading to poor pregnancy outcomes.

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CLINICAL CORRELATES OF MAGNETIC RESONANCE IMAGING IN PEDIATRIC CEREBRAL MALARIA

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Elucidating the pathogenesis of pediatric cerebral malaria (CM) has been a challenge for clinicians and pathologists because the relative contributions of sequestered parasites, metabolic derangements, seizures, and co-infections have been difficult to determine. The routine clinical characterization of pediatric CM patients in Blantyre includes direct and indirect ophthalmoscopy (to identify patients with malaria retinopathy), routine electroencephalography (to describe seizure semiology and identify subclinical seizures), and blood and cerebrospinal fluid cultures (to identify co-infections). The recent addition of neuroimaging, using a 0.35T Signa Ovation magnetic resonance imaging (MRI) machine (General Electric), has facilitated the recognition of previously unrecognized neuroradiologic features. Two-hundred thirty one patients with clinically defined cerebral malaria have undergone MRI imaging; the overall mortality rate was 13.4%. Of those scanned, 169 had malaria retinopathy, and 28 (16.6%) of these died. When compared to patients with retinopathy-negative CM (e.g., patients with a non-malaria cause of coma and parasitemia), the retinopathy-positive CM patients were more likely to have cerebral edema, and signal changes in the periventricular white matter, the basal ganglia and the corpus callosum. Frequently, rapid and significant changes were observed within 24-72 hours of admission. Seventy-three CM patients with retinopathy (43%) had evidence of severe cerebral edema (effacement of sulci, evidence of herniation) on admission. Twenty-eight (37%) of these patients died. There were no deaths in retinopathy-positive CM patients without evidence of severe brain swelling. These findings suggest that cerebral edema is an important feature in children with cerebral malaria, and that measures targeted at quickly reducing brain swelling may have an impact on survival.

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TRANSCRIPTIONAL PROFILING OF *PLASMODIUM FALCIPARUM* PARASITES FROM PATIENTS WITH SEVERE MALARIA: PARASITEMIA DRIVEN EXPRESSION AND UNIQUE BIOLOGICAL STATES

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The transcriptional biology of *Plasmodium falciparum* parasites from severe malaria *in vivo* has not been described. We collected peripheral blood from children meeting the clinical case definition of cerebral malaria and performed whole genome *ex vivo* transcriptional profiling for *Plasmodium falciparum* using a custom designed Affymetrix array. After normalization and clustering, two distinct biological clusters were observed that strongly correlated with the level of parasitemia observed in the patients at the time of collection. The low parasitemia cluster A (a heterogeneous mixture of profiles) showed upregulation of cell adhesion molecules, the trophozoite and gametocyte stages of the parasite, glycolysis, and cell cycle/DNA replication while the high parasitemia cluster B (a collection of very similar homogeneous profiles) demonstrated upregulation of genes and pathways associated with the ring stage of the parasite, activation of the ubiquitin pathway, and cytoplasmic ribosome translation. Comparisons with previous *ex vivo* transcriptional data in Senegal (predominantly from low parasitemia patients) showed expected overlap with cluster A while cluster B appears to be similar but unique to the Malawi patients. When ~1400 expression experiments from yeast were projected onto the Malawi metagenes, a subset of the Cluster B parasites could not be matched with known yeast biology although overlap with existing drug experiments were found. Correlation with the available clinical data demonstrated higher hematocrit in cluster A while the cluster B showed a higher use of mosquito repelling bed nets suggesting a link to low parasitemia. Lastly, we compared retinopathy the cluster A and B patients with retinopathy (and thus cerebral malaria) to all of the retinopathy negative patients (a heterogeneous mixture of diagnoses) and found the same association with parasitemia but also with white cell count (borderline significant); there were, however, a set of clinical differences that were distinct for retinopathy positive and not explained by parasite expression biology including low platelet count (expected), increased HIV rate, low hematocrit/hemoglobin (expected), low glucose (expected), high rate of bednet use, male gender (expected), and higher rate of lumefantrine/artemether use.

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MIXED INFECTION (*PLASMODIUM FALCIPARUM* AND *P. VIVAX*) DOES NOT REDUCE THE SEVERITY OF THE *P. FALCIPARUM* MALARIA - A STUDY FROM BIKANER, NORTHWEST INDIA

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Malaria remains a major health concern in tropical and subtropical countries. The last decade has witnessed a changing pattern of presentations and complications across the world. Independently *Plasmodium falciparum* malaria as well as *P. vivax* are known to cause of severe malaria and multiple organ failure whereas the effect on severity in mixed infection is conflicting. This prospective observational study describes the severity pattern and spectrum of severe manifestation (SM) of *P. falciparum* (Pf) mono infection as well as mixed infection with *P. vivax* (Pv+Pf) and study the effect of concomitant *vivax* infection on severity. This observational study included 887 adult patients amongst them 781 (88.04%) were Pf, and 106 (11.95 %) were mixed infection admitted in medical wards of S.P. Medical College and Associate Group of Hospitals, Bikaner, India from Sept. 2007 to December 2010. Species diagnosis was done by PBF and RDT. PCR confirmation on 100% patients revealed >96% accuracy. As defined in WHO criteria (2000) severe malaria was detected in 508 (57.27 %) patients with relative risk in Pf as 56.72 % (443/781) and in mixed infection as 61.32 % (65/106) [Pf - mixed infection p <0.0001)]. Hepatic dysfunction was the major SM (45.60% in Pf and 49.23% in mixed infection), followed by severe anemia (39.95% in Pf and 35.38% in mixed malaria), renal failure (12.19% in Pf and 10.77% in mixed malaria), cerebral malaria (8.80% in Pf and 10.77% in mixed malaria) and ARDS (0.68% in Pf and 0.0% in mixed malaria). Thrombocytopenia was observed in 46.05% in Pf and 50.77% in mixed infection. Multi organ dysfunction was 43.12% in Pf and 63.08% in mixed infection (p<0.0001). Mortality in indoor patients was 2.43 % (19/781) in Pf and 5.66% (6/106) in mixed infection. Inter group differences in all manifestations were statistically not significant but in case of MODS it was highly significant (p<0.0001). In conclusion, mixed infection does not reduce the severity of Pf malaria; rather it increases the severity and mortality.

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DISSECTING THE *PLASMODIUM FALCIPARUM* EVASION OF THE MOSQUITO IMMUNE SYSTEM

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Anopheles gambiae, main vector of malaria in Africa, varies in its susceptibility to different *Plasmodium falciparum* lines. It has been found that the mosquito innate immune system through the TEP1 pathway is able to eliminate *Plasmodium* parasites. We studied the role of the TEP1 pathway in determining the susceptibility of *Anopheles gambiae* to different *P. falciparum* lines. The *A. gambiae* L3-5 presented high susceptibility to *P. falciparum* 3D7, NF54 and GB4 lines, but it encapsulated in the midgut 97% of *P. falciparum* 7G8 parasites. dsRNA mediated knock down of components of the TEP1 pathway (TEP1, LRIM1, and APL1) rescued the 7G8 line from encapsulation, indicating that this pathway determines its elimination. On the other hand, dsRNA silencing of TEP1 and LRIM1 did not change significantly the infection of L3-5 mosquitoes with the *P. falciparum* NF54 line, indicating that this line evades the TEP1 pathway. Coinfection of *A. gambiae* L3-5 with a *P. falciparum* line that is encapsulated (7G8) and one that infects the mosquito effectively (3D7) led to mosquito midguts with both live and encapsulated parasites without any outcome dominating over the other. This indicates than even with activation of the TEP1 pathway by a *P. falciparum* line, a genetically different parasite can still evade it, suggesting that the evasion mechanism is parasite specific and not systemic in nature. Infecting with *Plasmodium* an extensive genetic cross of *A. gambiae* L3-5 with G3 *Plasmodium* susceptible mosquitoes we found that while the encapsulation of *P. berghei* is still mainly determined by the TEP1 alleles present in the mosquito, the *P. falciparum* 7G8 is no longer encapsulated, indicating that there are other factors besides the TEP1 allele that determine whether *P. falciparum* is eliminated by the mosquito immune system. The evasion of the *A. gambiae* immune system may be the result of adaptation of the parasite to the mosquito and has important implications for understanding disease transmission and the possibility of controlling it by enhancement of the TEP1 pathway in the mosquito.

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ANCESTRAL CHROMOSOMAL ARRANGEMENT AND REVISED PHYLOGENY OF THE *ANOPHELES GAMBIAE* COMPLEX

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The *Anopheles gambiae* complex consists of seven morphologically indistinguishable sibling species. Members of the complex have different geographical distribution, behavior and vectorial capacity, however, the phylogenetic relationship among the members of the complex is not resolved. For a long time *An. quadriannulatus* was considered ancestral because of its central position relative to other species, later analysis of the 2La inversion revealed that this arrangement is ancestral and has a unique origin. As a result *An. gambiae*, *An. arabiensis* or *An. merus* that carry this inversion could be closest to ancestral species. In this study, the breakpoints of 2Rop, the inversions fixed in *An. merus,* have been analyzed. Genes adjacent to breakpoints were identified by screening the *An. merus* Lambda Dash II phage library, Fluorescent *In Situ* hybridization (FISH), and Mate- Pair genome sequencing of *An. merus*. Proximal breakpoint of 2Ro inversion was obtained by screening the *An. merus* phage library and the distal breakpoint of 2Ro inversion was obtained by Mate -Pair sequencing of *An. merus* genome. FISH was done with the genes adjacent to breakpoints. The gene structure of inversion breakpoints was compared with several outgroup species including *An. stephensi, An. nili, An. moucheti, An. sinensis, Aedes aegypti* and *Culex quinquefasciatus.* The same gene arrangement at the 2Ro breakpoints was found in outgroup species, confirming the ancestral status of the 2Ro inversion. FISH also showed the same gene arrangement in 2Rp breakpoints in *An.gambiae* and outgroup species indicating that 2Rp+ arrangement is ancestral. Based on our data, we revised the chromosomal phylogeny of *An. gambiae* complex. We conclude that since 2La, 2Ro and 2Rp+ arrangements are ancestral; a hypothetical species that contained all these arrangement could be ancestral. It could have given rise to *An. merus* by obtaining the 2Rp arrangement and gave rise to *An. gambiae* by acquiring the 2Ro+ arrangement. The data suggest that the major vector of malaria in the world is more closely related to ancestral species and evolution shows that vectorial capacity can be lost in other members of the complex.

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A CONTINENT-WIDE MICROSATELLITE SURVEY REVEALS FURTHER COMPLEXITIES IN THE POPULATION STRUCTURE OF *ANOPHELES GAMBIAE* S.S. (*DIPTERA: CULICIDAE*)

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The major malaria vector *Anopheles gambiae s.s.* displays strong population subdivision across sub-Saharan Africa. In West Africa, two molecular forms have been described and are considered units of incipient speciation, albeit with varying levels of inter-form gene flow along their sympatric distribution range. However, studies on molecular form differentiation often analyzed samples from relatively few localities or regions. To provide an overall picture of the population structure of *An. gambiae s.s.* in west Africa, we have genotyped 25 samples, obtained mostly by indoor resting collections, from 12 African countries for 13 microsatellites on chromosome-3. Our area-wide results confirm a clear genetic differentiation between M and S forms using loci outside genomic regions of highest divergence. Furthermore, both Bayesian clustering and principal components analyses revealed further population substructuring in the M-form, with samples from Western Africa (from The Gambia to Nigeria) forming a distinct genetic cluster from those of West-Central Africa (from Cameroon to Angola). This subdivision is likely to be associated with the Forest-savannah ecosystem transition coupled with the accumulation of polymorphic chromosomal inversions in this vector species.

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CHROMOSOMAL AND MOLECULAR CHARACTERIZATION OF *ANOPHELES GAMBIAE* M AND S MOLECULAR FORMS IN A SECONDARY CONTACT ZONE AT THE WESTERNMOST EXTREME OF THEIR RANGE

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Throughout west and central Africa, *Anopheles gambiae* M and S molecular forms are characterised by largely overlapping geographical/ temporal distributions and high levels of reproductive isolation. Floating paracentric inversions on chromosome-2, probably involved in ecological adaptation to marginal sub-niches, are shared by the two forms, although with different frequencies of alternative inverted arrangements, reaching the highest level of inter-form differentiation in northern savannah areas. At the westernmost extreme of their range, however, a secondary contact zone between M and S forms has been recently revealed based on the finding of putative M/S hybrid frequencies higher than in the rest of the form range (i.e. 3-7% in The Gambia and >20% in Guinea Bissau). We here report the first results of the karyotyping of M and S populations collected in two west to east transects: one along the Gambia river in The Gambia and eastern Senegal and one from the capital city eastwards in Guinea Bissau. The results show that in coastal and central Gambian areas, as well as in the Guinean capital city area, M and S populations are found in sympatry and share the same chromosomal polymorphisms based on 2Rd and 2La inversion. On the other hand, in Senegalese sampling sites, S-form is largely predominating over M-form and is characterised by increasing frequencies of 2Rj, 2Rbk and 2Rcu inverted arrangements, while in eastward Guinean sites, S-form characterized by increasing frequencies of 2Rj and 2Rb is virtually the only form found. We will couple these results with those obtained from an extensive microsatellite analyses carried out on the same samples in order to provide a more detailed picture of the genetic differentiation between the two molecular forms in this area of secondary contact at the westernmost extreme of their range and to speculate on the genetic adaptive mechanisms allowing *An. gambiae* s.s. great ecological flexibility.

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WING SIZE DIFFERENTIATION BETWEEN THE INCIPIENT SPECIES OF *ANOPHELES GAMBIAE* S.S. AND ITS POTENTIAL ROLE IN ASSORTATIVE MATING

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Anopheles gambiae s.s., one of the most important malaria vector species in Africa, has been subdivided into two molecular forms, M and S, which are thought to represent incipient species. The two forms have varying levels of phenotypic and genetic divergence in different locations within their range. Wing beat frequency may provide the necessary phenotypic information required to explain the assortative mating observed between forms. Because wing size relates directly to both wing beat frequency and individual fitness we investigated the potential that wing size may vary with environmental and genetic variation in *An. gambiae* s.s in west Africa. We assessed the size of wings (wing length and wing width) from female mosquitoes collected at sites in Mali where a rarity of hybrids indicates strong between-form assortative mating, as well as Guinea-Bissau where high levels of hybridization suggest reproductive isolation between forms has broken down. We observed a significant difference in length and width of the wings between Guinea-Bissau and Mali. Guinea-Bissau mosquito wings were significantly smaller than those from Mali, regardless of molecular form. While we found no significant difference in mean wing length between molecular forms, we did find the S form to have significantly larger wing widths than the M form in Mali. By contrast, we did not observe this difference in Guinea-Bissau where the rate of hybridization in the field is high and assortative mating appears to be distorted. These data represent the first documentation of a morphological difference within *An. gambiae* s.s., between the two molecular forms. The significant difference observed between molecular forms for wing size, in an area of low hybridization and the lack of difference in an area of high hybridization supports the hypothesis that wing size and hence wing beat frequency may confer the necessary phenotypic information to account for the assortative mating observed with molecular data, between the molecular forms of this species.

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GENETIC ISOLATION BETWEEN *ANOPHELES MELAS* POPULATIONS

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Anopheles melas is found breeding in brackish waters along the coast of West-Africa. Because its distribution is limited to coastal regions, it is not often considered an important malaria vector, even though it is frequently the most abundant vector in those locations were it is present. Therefore, the species has not been studied well and little is know about its population structure. On Bioko Island, Equatorial Guinea, *An. melas* is an important vector. It is dominant in several locations where it is responsible for a high entomological inoculation rate. Malaria vector populations on Bioko have been targeted by anti-vector interventions implemented under the Bioko Island Malaria Control Project (BIMCP). As part of an operational research project under the BIMCP, we have investigated the population structure of *An. melas* on Bioko and mainland Africa to determine the level of migration between various populations on the mainland and Bioko Island. We have analyzed microsatellite data and mtDNA from 11 *An. melas* populations across West-Africa. We found that *An. melas* populations for the most part cluster into three distinct groups with a very high level of genetic differentiation between them. Populations on Bioko Island are almost completely isolated from the mainland. Additionally, mainland populations are divided into two distinct groups which do not share mtDNA haplotypes and little genetic exchange is evident in the microsatellite data. In fact, the level of mtDNA differentiation between the two mainland *An. melas* clusters is on par with that between *An. melas* and *An. gambiae.* These results indicate that *An. melas* on the mainland may consist of two previously undescribed species. Additionally, in contrast to its sister species *An. gambiae,* little or no migration exists between *An. melas* populations on the African mainland and Bioko Island.

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GENOME-WIDE PROFILING OF CIRCADIAN AND LIGHT-REGULATED GENE EXPRESSION OF THE *ANOPHELES GAMBIAE* MOSQUITO REVEALS DAILY RHYTHMS IN METABOLISM, DETOXIFICATION, IMMUNITY AND SENSORY PROCESSES

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Anopheles gambiae, the primary African mosquito vector of malaria, exhibits numerous rhythmic behaviors including flight activity, swarming, mating, host seeking, egg laying and sugar feeding. However, little work has been performed to elucidate the molecular basis for these daily rhythms. To study how gene expression is globally regulated by light and circadian mechanisms, we have undertaken a microarray analysis of *A. gambiae* under light:dark cycle (LD) and constant dark (DD) conditions. Adult female mosquitoes were collected every 4 hr over 48 hr and samples were processed with microarrays. Using a cosine-wave fitting algorithm, we identified 1293 and 600 rhythmic genes with a 20-28 hr period length in the head and body under LD conditions, representing 9.7 and 4.5% of the *A. gambiae* gene-set (www.nd.edu/~bioclock). A majority of these genes was specific to heads or bodies. Through examination of mosquitoes under DD conditions, we reveal that rhythmic programming of the transcriptome is dependent upon an interaction between the endogenous clock and extrinsic regulation by the LD cycle. A sub-set of genes was rhythmically expressed under both environmental conditions, including the canonical clock components. A majority of genes had peak expression clustered around the day/night transitions, anticipating dawn and dusk. Genes cover diverse biological processes such as transcription/translation, metabolism, detoxification, olfaction, vision, cuticule-regulation and immunity. For example, 33% of cytochrome P450 genes are rhythmically expressed, including CYP6Z1, CYP6P3 and CYP6M2 that are implicated in pyrethroid resistance. In olfaction, genes encoding odorant binding proteins and the olfactory coreceptor OR7 are rhythmically expressed, as are components of the visual transduction pathway, suggesting that daily changes occur in sensory perception and in the ability of the mosquito to detect host-cues. This study highlights both the fundamental roles that the circadian clock and light play in the physiology of *A. gambiae*, and suggests novel targets for intervention.

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THE RESEARCH ON THE SCHISTOSOMIASIS TRANSMISSION DYNAMIC MODEL BASED ON ARTIFICIAL INTELLIGENCE

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Schistosomiasis is one of the most prevalent parasitic diseases worldwide, with 207 million people infected in 76 countries. In China, *Schistosoma japonicum* is the species that causes human infection and disease in endemic areas. Although over 60 years' control efforts has successfully bring down the prevalence, further reduction and eradication of the disease remain difficult due to the complexity of life cycle of the parasite as well as the impact of individual behavior on transmission dynamics. To better predict the impact of control policies including chemotherapy scheme and vaccination at the low-endemic setting, here we present a stochastic model (SjCA-Q) based on a revised cellular automata model using Q-learning algorithm as the artificial intelligence to describe the transmission dynamics of human schistosomiasis japonica in an endemic area in China. This model includes the process of pathogen invasion from exposure to worm development and worm death when the infection is cleared; it also incorporates seasonality of infection in the endemic field as well as the stochastic behavior of each individual. Q-learning algorithm is used for rules self-learning in the model. For simulation, we used data collected from a two-year longitudinal study conducted in Jiahu village on the southeastern shore of Poyang Lake in Jiangxi Province. We applied our model in evaluation of several current control strategies for schistosomiasis in China. A multi-dimensional evaluation system is used to judge the simulate result. Our model can effectively simulate the transmission process. The Q-learning algorithm enabled the model self-learn the uncertain rules in disease transmission model and significantly improve the simulation effect. Chemotherapy should cover no less than 85 percent of the *Schistosoma japonicum* infected population, and implemented twice a year in the low-endemic community to guarantee an effective control. In conclusion, it is anticipated that our SjCA-Q transmission model can serve as a tool for understanding schistosomiasis transmission dynamics and thus the knowledge learnt from modeling would be prerequisite for focusing and improving schistosomiasis control at the local level.

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NEW AND IMPROVED IMMUNOLOGICAL ASSAYS FOR DIAGNOSIS OF *SCHISTOSOMA MANSONI*

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Increasing populations and human migration are contributing factors in the observed increases of *Schistosoma mansoni* in new areas of southeastern Brasil. Control constraints include the lack of diagnostic methods with high sensitivity. We initiated a study in southeast Brasil to develop and improve diagnostic methods for *S. mansoni*. Individuals from 3 endemic areas and, non-endemic rural tourists with acute disease were selected as sera and feces donors. Specificity/sensitivity of new diagnostic methods tested were compared to results obtained from 20 Kato-Katz prepared slides from 3 different fecal samples collected on different days for each individual. Miracidial hatching from each fecal sample was also measured. We evaluated the efficiency of egg versus worm antigens using two immunosorbant assays for IgG detection based on the antigens being highly immunogenic and easily obtained. Both new tests presented a kappa index of 0.46. The first new assay had sensitivity/specificity of 75% with a cut off of 0.31. The second new assay had 100% and 97% sensitivity/specificity respectively with a cut off of 0.18. Currently, diagnosis of pre-patent schistosome infections is difficult due to non-specific symptoms. Therefore, we standardized a new IgG detection assay using schistosomula antigen. Data showed excellent agreement of kappa index (0.82) and 91% and 53% of sensitivity/specificity with a cut off of 0.29, including all chronic patients with low parasite load (1-200 epg/feces). The method was also able to detect high antibody titers in sera of non-endemic patients after 7 days of the infection. Although antibody-based methods suffer from low sensitivity, especially for acute phase, we were able to identify positive cases for endemic and non-endemic patients. Finally, we developed a direct method for use with acute and chronic phase diagnosis that differentially detects *Schistosoma* antigens from other worms using low amounts of human sample.

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ASSESSMENT OF SUBTLE MORBIDITY DUE TO SCHISTOSOMIASIS AMONG SCHOOL CHILDREN: THE SCORE PROJECT IN KENYA

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Chronic *Schistosoma mansoni* infection is associated with liver fibrosis, stunting, wasting, anemia, exercise intolerance, and decreased quality of life. Much of the morbidity associated with schistosomiasis is subtle, making it difficult to quantify disease burden. Thus, the effectiveness of control programs may be underestimated. The Schistosomiasis Consortium for Operational Research and Evaluation (SCORE) project is a five year multi-national study designed to evaluate optimal control strategies for *S. mansoni* associated morbidity. We present the merged baseline results of morbidity assessments in two cohorts from Kenya. A total of 817 children aged 7-8 years near Kisumu, Kenya were randomly selected from 12 schools with *S. mansoni* prevalence ≥25%. Stool was collected to test for *S. mansoni* infection and intensity, and soil-transmitted helminth (STH) infection. Blood was tested for malaria and anemia status. Ultrasound data were collected. Stool results from 620 persons show a prevalence of *S. mansoni*, *Trichuris trichiura*, *Ascaris*, and hookworm of 66.2% (62.4-70.0), 15.7% (13.1-18.8), 12.0% (9.7-14.9), and 5% (3.5-7.1), respectively; 26.8% (23.4-30.4) were positive for any STH, and 73% (69.4-76.4) were positive for at least one helminth. Malaria infection was found in 8.0% (5.8-11.0) of the 424 tested. Anemia was present in 48.3% (44.9-51.8) of the 801 children tested- 40% (36.6-43.4), 7.6% (6.0-9.7), and 0.8% (0.3-1.7) with mild, moderate, and severe anemia, respectively. Abdominal ultrasounds showed abnormal liver texture patterns in 23.6% (20.7-26.7) of the 781 children tested. Univariate analysis of infection and anemia showed statistically significant associations with *S. mansoni*, OR 1.5 (1.04-2.1), and any helminth infection, OR 1.46 (1.01-2.12) only. In this cohort of 7-8 year old Kenyan children, *S. mansoni* and STH infections were highly prevalent and associated with anemia. Dataset completion and multi-variate analyses, including exercise tolerance, anthropometric, and quality of life measures, are forthcoming.

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ASSESSMENT OF QUALITY OF LIFE AS A TOOL FOR MEASURING MORBIDITY DUE TO SCHISTOSOMIASIS AND THE IMPACT OF TREATMENT

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Schistosomiasis control programs are designed to reduce morbidity associated with the infection. In areas endemic for *Schistosoma mansoni*, change in prevalence, based on stool examination, is the primary

method used for monitoring the efficacy of treatment. However, this assessment is often difficult to conduct and may not reflect the health benefits gained. Because schistosomiasis can persist for many years causing unrecognized morbidity, a proposed approach to assess program impact is to use questionnaires that capture measures of quality of life. To evaluate whether the short form WHO quality of life assessment (WHOQOL-BREF) is useful to measure the benefit of treating *S. mansoni* infections, adults from a highly endemic area (> 75% prevalence) who had no recollection of prior treatment with praziquantel were enrolled. Prior to treatment, the WHOQOL-BREF was administered to non-pregnant, consenting participants who were evaluated for schistosomiasis by stool exam, serum antibody levels, and presence of circulating cathodic antigen (CCA) in urine. Additionally, participants were tested for infection with malaria, soil transmitted helminths and HIV. At baseline, there was no association between schistosome infection status or intensity of infection and quality of life. Urine CCA levels were significantly reduced within 2 days of treatment. Six months after treatment, the WHOQOL-BREF was administered again and stool and urine samples collected. There was a significant reduction in both prevalence and intensity of infection and quality of life significantly improved compared to baseline. However, persons who did not have detectable *S. mansoni* infections at baseline demonstrated similar improvements in their WHOQOL-BREF scores as did persons infected at baseline. Similarly, there was no relationship in the baseline intensity of infection and the improvement of reported quality of life. Thus, in areas of high prevalence and intensity of schistosomiasis, the WHOQOL-BREF may not be able to specifically detect the benefits of mass drug administration programs.

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COMPARISON OF QUESTIONNAIRE-BASED PRAZIQUANTEL TREATMENT HISTORY WITH HEMATURIA AMONG SCHOOL CHILDREN

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National schistosomiasis control programs often report the number of praziquantel (PZQ) treatments distributed as a measure of program output, along with coverage calculations that use a host of different denominators, including total population, eligible population, or census population. It has been proposed that reported coverage by programs be independently confirmed through individual responses in drug coverage surveys. However, response bias and/or recall bias have been proposed as major limitations of such surveys. As part of a 2010 evaluation of ongoing PZQ distribution to school-aged children in Plateau and Nasarawa states of north-central Nigeria, we randomly selected 482 school-children 10-14 years of age from 12 schools across two LGAs receiving annual PZQ treatment to reassess hematuria using urine reagent dipsticks. Baseline mean hematuria in these village in 2008 was 34.1%. All students were asked whether they took or did not take PZQ for schistosomiasis in the last year. PZQ tablets were shown to the children upon assessment. Overall, 13.3% (6.9-19.7%) tested positive for hematuria. Drug coverage as reported by surveyed children was 64.5% (47.3-81.8%). After controlling for sex, baseline community prevalence and reported knowledge of schistosomiasis, the odds of having hematuria were 2.1 (95%CL 1.13-3.9) times higher among children reporting not taking PZQ than children reporting taking PZQ. These results suggest school children provide reliable history of PZQ treatment. National programs should consider implementing similar evaluation surveys in schools to monitor drug coverage and impact.

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COMMUNITY-BASED INTERVENTION TO REDUCE INCIDENCE OF SCHISTOSOMIASIS THROUGH TREATMENT AND HEALTH EDUCATION IN RURAL AFRICA

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Schistosomiasis is a chronic parasitic infection affecting millions of Africans, acquired in fresh water via snail host. This is particularly precarious for inhabitants of rural areas that depend on the local fresh-water source. Due to limited access to hospitals and education, lakeside villagers are untreated, and simply unaware of precautions against schistosomiasis. By providing annual on-site screening, education and treatment, the incidence of Schistosomiasis can be reduced. Since 2006, a longitudinal study has been carried out in Minigo, a village along Lake Victoria. Minigo (pop. 3635) was educated on the practices exposing them to Schistosomiasis and how to modify daily activities to minimize this. On location screening for *S. haematobium* and *S. mansoni* was provided through physical exam and fecal and urine sample testing. Praziquantel 40 mg/kg was prescribed to those who tested positive and prophylactically for fisherman. For those with complications, an ultrasound was provided to determine need for other treatment. For those positive for other infections, appropriate treatment was given. In 2010, this method was introduced into a second village, Masonga (Pop. 2000). In Minigo, the incidence was 30% in 2007, 14% in 2009, and 10% in 2010, yielding a 67% decline. Masonga (Pop. 2000), providing a basis for comparison, had an incidence of 20% in 2010. While all of Minigo and Masonga had access, only 5% and 10%, respectively, elected to be screened and treated. Accordingly, the prevalence of Schistosomiasis can be effectively reduced by providing access to treatment and preventative education at the community level. Masonga, providing a baseline rate two times higher than that of Minigo reiterates the value of this intervention. Increasing participation is needed for the eradication of Schistosomiasis, but the factors influencing decisions to seek community-based disease prevention has not yet been determined. If these results are confirmed with the continuation of this study, this method of cooperative intervention will provide a feasible model for Schistosomiasis reduction in lake-dependent villages in rural Africa.

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BILHVAX, A VACCINE CANDIDATE AGAINST SCHISTOSOMIASIS

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The development of an efficient vaccine against human schistosomiasis represents a major challenge for the improvement of health in many developing countries where continuous re-infection point to the necessary development of alternative strategies to chemotherapy. In schistosomiasis, where parasite eggs laying in the tissues is the exclusive cause of pathology and the elimination of eggs in nature is the source of transmission, inhibition of parasite fecundity might represent a way to prevent the deleterious effects of these chronic infections in man. The concept to target by vaccination the cause of the pathology rather than the parasite itself would provide a potent tool to control a major chronic infection. In *Schistosoma haematobium* infected children, clinical trials (Phases I-II) provided evidence of a safe and immunogenic molecule inducing in man a profile of immune response which seems in accordance with the experimental models describing the effect on inhibition of female worm fecundity and egg viability. On this basis, Inserm proposed to develop efficacy phases (Phases III). As a whole, the first step of clinical trials of the first vaccine candidate against human schistosomiasis Phase III trial, self-contained, randomized, double blind, in two parallel groups receiving 3 primo-vaccinations and a boost has been stated in March

2009 for a three-year trail. During this trial, one group of *S. haematobium* infected children (6 to 9) receives "Bilhvax", the other one, placebo. Both groups were treated with Praziquantel. The aim of the trial is to evaluate efficacy and safety of the therapeutic vaccine candidate Sh28GST in association with Praziquantel for prevention of clinical and parasitological recurrences of *S. haematobium* infection in children. These trials which are performed in already established clinical platform in Senegal and could be easily extended to other schistosome sp infections, represents a crucial step in innovative approaches to solve persistent problems of these chronic parasitic infections.

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PLASMODIUM FALCIPARUM HISTONES INDUCE ENDOTHELIAL PRO-INFLAMMATORY RESPONSE AND BARRIER DYSFUNCTION

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Severe *Plasmodium falciparum* infection is associated with endothelial activation and permeability that are important determinants of the outcome of the infection. How endothelial cells become activated is not fully understood but is believed to be either a response to cytokines from leukocytes and/or a direct effect of parasite components. In this study, we demonstrated that *P. falciparum* sonicates directly stimulated the production of IL-8 and other inflammatory mediators by primary human dermal microvascular endothelial cells through a signalling pathway that involved the Src family kinase Lyn and p38 MAPK. The active parasite component was identified as acid soluble proteins (HeH) of which histones were a major constituent. The role of histones was confirmed by abrogation of the stimulatory effect of HeH by histone-specific antibodies and the use of recombinant *P. falciparum* H3 (PfH3) and recombinant human H4. Confocal microscopy of methanol-fixed blood smears revealed that prior to schizogony, histones could be seen both inside and outside of nuclei of merozoites. The release of nuclear contents upon IRBC rupture was captured by live cell imaging using the cell membrane impermeable DNA stain Sytox Green, and was confirmed by detecting nucleosomes in supernatants of parasite cultures. HeH and recombinant histones also induced endothelial permeability through a charge-dependent mechanism that resulted in disruption of junctional protein expression and cell death. Recombinant human activated protein C cleaved HeH and PfH3 and abrogated both IL-8 production and increased permeability. Circulating nucleosomes of both human and parasite origin were detected in the plasma of patients with *falciparum* malaria, and correlated positively with disease severity. These results strongly support a pathogenic role for both host- and pathogen-derived histones in *P. falciparum* malaria.

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THE UNUSUAL TCA METABOLISM IN *PLASMODIUM FALCIPARUM*

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The role of tricarboxylic acid (TCA) metabolism in malaria parasites has been poorly understood for decades. Recent studies have shown that glutamine and glutamate are the primary carbon sources of the TCA metabolism and that the TCA "cycle" splits at α-ketoglutarate, forming a branched architecture. The functions of this unique TCA metabolism in *Plasmodium* parasites have been proposed to provide precursor for heme biosynthesis and to generate acetyl-CoA (instead of consuming it). To investigate this altered TCA metabolism, we have generated a series of knockout mutants via homologous recombination strategies. We have individually knocked out genes encoding α-ketoglutarate dehydrogenase (KDH), succinyl-CoA synthetase (SCS), succinate dehydrogenase (SDH) and isocitrate dehydrogenase (IDH). Furthermore, we generated KDH/SCS and SCS/SDH double knockouts. Remarkably, none of the knockout lines exhibited growth defects, suggesting these enzymes to be nonessential in the blood stage. In particular, the viability of the KDH/SCS double-knockout line indicates that succinyl-CoA production from the TCA metabolism is not essential. This result challenges the long held notion that TCA metabolism is necessary to provide the essential precursor (succinyl-CoA) for heme biosynthesis. Fumarate hydratase could not be deleted, suggesting it is essential. To determine the metabolic consequences of knocking out these TCA enzymes, we incubated the knockout parasites with U-^{13}C-glutamine medium. Oxidative and reductive TCA fluxes were quantified by measuring differential isotopic enrichment in various TCA intermediates by liquid chromatography mass spectrometry (LC-MS). ΔSCS line had no significant changes in the TCA metabolites. All ΔKDH and ΔSDH lines (single and double knockout) showed significantly diminished oxidative TCA flux when compared to wild type. As expected, reductive flux in the ΔKDH and ΔSDH lines was comparable to wild type. In contrast ΔIDH line showed significant impairment in both oxidative and reductive TCA fluxes, indicating little to no TCA cycle activity in these parasites. Surprisingly, such changes do not affect parasite survival, indicating a high degree of metabolic flexibility in malaria parasites.

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INTERFERON REGULATORY FACTOR 8 (IRF8) REGULATED PATHWAYS: ACTIVE ROLE IN PATHOGENESIS OF CEREBRAL MALARIA BUT REQUIRED FOR PROTECTION AGAINST TUBERCULOSIS

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Interferon Regulatory Factor 8 (Irf8) is a transcription factor that plays an important role in myeloid cell development and function. It is not only required for the development of monocytes, macrophage and dendritic cells, but also for transcription of intrinsic (microbicidal effector proteins) and extrinsic (IL-12p40) defense mechanisms expressed by these cells. The BXH2 mouse strain harbors an *Irf8*^{R294C} hypomorphic loss-of-function allele. BXH2 mice, or myeloid cells derived from them, are susceptible to many infections including *Mycobacterium bovis* BCG, *M. tuberculosis*, and *Legionella pneumophila*. Here, we observe that BXH2 mice are highly resistant to cerebral malaria caused by infection with *Plasmodium berghei* ANKA. Transcriptional profiling of BXH2 total brain RNA following *P. berghei* infection shows that CM-resistance in these mice is associated with the failure to transcriptionally activate interferon responsive inflammatory pathways. Of the 55 genes up-regulated (>2.5-fold) in cerebral malaria in an Irf8-dependent fashion (present in susceptible C57BL/6 and significantly reduced in BXH2 mice) following *P. berghei* infection, several of them are known to be play critical roles in Th1 polarization of immune response, including Irf1 and Stat1. Seventeen of these genes were also up-regulated by a factors of >1.8-fold in C57Bl/6J mouse lungs infected with *M. tuberculosis*. 94% (16/17) of genes regulated by both infections contained an Irf8 binding site, as determined by chromatin immunoprecipitation in macrophages followed by genome-wide hybridization (ChIP-chip). Additional studies in Stat1, Irf1 and If knockout mice confirmed that disruptions in this pathway confer both CM-resistance and susceptibility to tuberculosis. Our findings indicate that a robust Th1 response plays an important detrimental role in pathogenesis of cerebral malaria, while being required for protection

against mycobacterial infections. The Irf8-dependent transcriptional program identified here is shown to play a pivotal role in this response; Irf8 and/or members of this pathway may constitute a potential therapeutic target for intervention in cerebral malaria.

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ACTINOMYCETE-DERIVED INHIBITORS OF FILARIAL ASPARAGINYL-TRNA SYNTHETASE

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Aminoacyl tRNA synthetases (AARS) were one of the first new molecular targets embraced by the W.H.O helminth drug discovery program and are generally regarded as excellent therapeutic targets because; (i) they perform important primary and secondary transformations within eukaryotes including filaria and other human and veterinary parasites, (ii) are essential to parasite viability, and (iii) demonstrate primary and secondary structural heterogeneity. Among the AARSs AsnRS (asparaginyl-tRNA synthetase) is an excellent filarial target because (i) it is expressed in both sexes, adults, L1 (bloodborne microfilariae) and L3 (infective) larvae of *Brugia malayi* and *Wuchereria bancrofti*, (ii) is well-characterized biochemically and structurally, and (iii) recombinant *B. malayi* AsnRS is amenable to overexpression and use in high throughput bioassay-guided screening algorithms. As part of a drug discovery program targeting the *B. malayi* AsnRS we recently screened ~73,000 microextracts from a collection of 36,720 microbial strains for activity against *B. malayi* AsnRS. Natural product producers evaluated included members of the *Streptomyces, Deuteromyces, Aspergillus, Euteromyces, Peniclium, Malbranchea, Fusarium* and *Mucor* species. We recently reported that one of these strains, *Streptomyces* sp. 17944, produces a tirandamycin (TAM) with the ability to inhibit filarial AsnRS and rapidly kill adult worms. New data is now presented on optimization of experimental conditions that facilitate production of the TAM and application of these methods to discovery of additional filarial AsnRS inhibitors. We have identified fermentation conditions affording the TAM as the major product (with titers ~ 12 mg/L) and we have a developed an expedient genetic system for manipulation of TAM biosynthesis in *S*. sp. 17944. These results (i) demonstrate the feasibility of *in vivo* manipulation of TAM biosynthesis in *S*. sp. 17944 and (ii) ensure that sufficient amounts of the TAM can be produced and isolated for proposed follow up mechanistic and preclinical studies for consideration as a novel antifilarial.

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STANDARDIZING THE MEASUREMENT OF PARASITE CLEARANCE: PARASITE CLEARANCE ESTIMATOR

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The emergence and spread of resistance to antimalarial drugs threatens the efficacy of existing drug treatments. Although guidelines do not currently exist that define a consistent method of identifying resistance, monitoring times to parasite clearance has been widely used. The pharmacodynamic hallmark of the artemisinin derivatives is that they give more rapid parasite clearance than other antimalarials. Thus, accurate measurement of parasite clearance is critical to assess the emergence and spread of artemisinin resistance in *Plasmodium falciparum*, which has recently emerged in Western Cambodia. After starting antimalarial treatment, a lag phase of numerical instability, often precedes a fall in the parasite count. This complicates the parasite clearance rate estimation, introduces observer subjectivity, and may influence both the accuracy and consistency of results. To address this problem, a new approach to modeling clearance of parasites has been explored and validated. This model detects when a lag phase is present, allows the best model to be chosen from log linear, quadratic and cubic fits and calculates estimates of parasite clearance adjusted for this lag phase. Parasite measurements below the level of detection are accounted for in the estimation, and not excluded, as is usual per standard practice. Based on data from clinical studies in South East Asia in which existing frequent parasite count data were obtained, we present individual patient data examples for which the lag phase has been identified and discuss the effect it has on clearance rate estimates. Goodness of fit and residual plots are compared between standard linear regression method and our lag phase method. As part of WWARN efforts to make innovative approaches available to the malaria community, we have developed an open access automated informatics tool. This tool provides a more accurate, consistent and improved method of estimating both the parasite clearance rate and the lag phase. It could be used to detect early warning signs of resistance to artemisinin derivatives.

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PLASMODIUM FALCIPARUM CLEARANCE RATES IN RESPONSE TO ARTESUNATE IN MALIAN CHILDREN WITH MALARIA

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Artemisinin resistance, currently defined as a slow parasite clearance rate (CR) *in vivo*, was recently described in Cambodians with *Plasmodium falciparum* malaria. Studies have not yet reported parasite CRs from Africa, where artemisinin-based combination therapies (ACTs) are first-line treatments for *falciparum* malaria. In this study we measured parasite CRs in 132 Malian children aged 1-15 years presenting with uncomplicated *falciparum* malaria in 2010. We provided directly observed weight-based doses of artesunate (days 0, 1, 2) and amodiaquine (days 3, 4, 5) orally to these children, and counted the peripheral blood parasite density every 6h until it was zero. From plots of log-transformed parasite densities *vs.* time we calculated the half-life of parasite clearance (the time it takes for parasite density to decrease by 50%) and evaluated the effects of age, sex, ethnicity and red blood cell (RBC) polymorphisms (sickle HbS, HbC, alpha-thalassemia, G6PD deficiency). We isolated parasites from 46 of these children and measured their *ex vivo* response to artesunate and dihydroartemisinin (DHA) in a conventional drug response assay. The mean (± SEM) half-life of parasite clearance was 1.99h +/- 0.068h and ranged from 0.4h to 5.3h. A linear regression analysis showed that the half-life of parasite clearance decreased with age, predicting a 0.1h reduction in half-life for every 1-year increase in age. *Ex vivo* IC50s for DHA, but not for artesunate, correlated positively with half-life of parasite clearance. These data indicate that the artemisinin resistance phenotype is not present in our study population, consistent with the very recent introduction of this drug at our study site. In high-endemic areas, analyses of parasite clearance in response to artesunate should account for age as a covariate, since acquired immunity may increase the rate of parasite clearance *in vivo*. Studies of artemisinin resistance in low-endemic areas like Southeast Asia may be confounded by the patient's level of acquired immunity.

We are now using this *in vivo* model of parasite clearance in response to artemisinin to identify IgG responses that correlate with the removal of *P. falciparum*-infected RBCs during the course of a malaria episode.

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PARASITE CLEARANCE TIME FOLLOWING ORAL ARTESUNATE TREATMENT OF UNCOMPLICATED *FALCIPARUM* MALARIA IN MALI, WEST AFRICA

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Artemisinin based combinations (ACTs) are currently the first line therapy for uncomplicated malaria. *Plasmodium falciparum* resistance to artemisinins, measured by parasite clearance time (PCT), has been reported in South-East Asia. Few data on PCT are available from Africa where malaria transmission is high, the burden of malaria is highest and the use of ACTs has been scaled up in the past few years. From December 2010 to February 2011, 100 children from Bougoula-Hamaeau, Mali aged 1-10 years with uncomplicated malaria were enrolled and treated with seven days of directly-observed oral artesunate after parental consent. Thick and thin blood smears were prepared and read every 8 hours for asexual and sexual parasite counts until three consecutive slides were negative. Patients were followed actively and passively for 28 days following a standard protocol. Results were compared to data from a similar study conducted in the same village by the same study team and during the same months in 2002/04. In the per protocol analysis, the uncorrected adequate clinical and parasitological response (ACPR) rate of 96.7% and corrected ACPR of 100% measured in 2010-2011 (n=91) were similar to those measured in 2002/2004(98.6% and 100% for uncorrected and corrected ACPR, respectively). The proportions of patients who cleared parasitemia by 24 hours after treatment initiation were 36.0% (n=92) in 2010/11 and 31.9% (n=72) 2002/2004 (p=0.5). The median PCT in 2010-2011 was 32 hours. No PCT could be calculated with the 2002/2004 data because slides were read only at 24-hourly intervals. To our knowledge, this is the first estimate of PCT after curative artesunate therapy in an area of high transmission of *falciparum* malaria. Artesunate was highly efficacious and we therefore provide a baseline PCT that is required for the surveillance of the efficacy of artemisinins on *P. falciparum* isolates from sub-Saharan Africa.

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EMERGENCE OF ARTEMISININ RESISTANCE ON THE THAILAND-BURMA BORDER

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Resistance to artemisinin (ART) in *Plasmodium falciparum* is suspected from foci in Western Cambodia and is characterized by slower parasite clearance rates (CR) following treatment with artesunate. However, there is little information on the distribution of this phenotype elsewhere in SE Asia, and the genotypic basis of this trait is unknown. We measured parasite CR (using 6 hourly measures of parasitemia following ART treatment) in 1733 hyperparasitemia patients from 4 clinics on the Thai-Burma border between 2001-2010, and genotyped all parasite infections with 93 polymorphic SNPs. Parasite CR decreased significantly between 2001-10. While 4% of patients showed CR < 0.15 in 2001, this increased to 45% in 2010, compared with 78% in Western Cambodia. At the current rate of decline CR on the Thai-Burma border will be indistinguishable from current rates in Western Cambodia in <5 years. Partner drugs cannot explain these patterns. We observed the same temporal changes in a subset of 874 patients treated with monotherapy for >48hrs prior to mefloquine treatment. There was a minor influence of patient age, but waning population immunity due to declining transmission was also insufficient to explain the changes observed. To examine the role of parasite genetics we identified identical 93-locus parasite genotypes infecting multiple patients. We identified 158 multilocus parasite genotypes each infecting 2 - 14 patients. In 2001-4 29% of variation in CR was attributable to parasite genetics. Interestingly, the two parasites with slowest CR during this period, both from 2003, had identical 93-locus genotypes, suggesting the presence of ART resistant parasites 8 years ago. By 2007-10 parasite genetic factors explained 64% of variation in CR, consistent with increasing frequencies of parasite alleles conferring ART resistance. Both epidemiology and genetics provide compelling evidence that parasite CR is declining on the Thailand-Burma border and that this is explained by parasite genetic factors present in an increasing proportion of the parasite population.

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ARC3: A GENOME-WIDE ASSOCIATION STUDY OF THE GENETIC BASIS OF PARASITE CLEARANCE RATE FOLLOWING TREATMENT WITH ARTEMISININS

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In the wake of widespread resistance to chloroquine and antifolate drugs, artemisinin-based combination therapies (ACTs) have been adopted as the first line treatment for *Plasmodium falciparum* malaria in most regions of the world. The successful use of ACTs with insecticide-

treated nets to dramatically reduce the malaria burden in some areas has sparked renewed consideration of a global malaria eradication campaign. The emergence of artemisinin-resistant *P. falciparum* in parts of western Cambodia threatens the recent major global investment in ACTs and prospects for eradication. As part of the Artemisinin Resistance Confirmation, Characterization, and Containment (ARC3) pilot project, four clinical trials of artesunate curative therapy were conducted at two sites in western Cambodia where emerging resistance was suspected; on the Thai-Myanmar border, where prolonged parasite clearance times following artesunate-mefloquine treatment had also been reported; and in Bangladesh, where ACTs have not been used extensively and resistance was not suspected. Parasites collected during these trials were genotyped at approximately 8,000 single nucleotide polymorphisms (SNPs) using a molecular inversion probe SNP chip specific to *P. falciparum*. Regression and Random Forests were used to associate parasite genotypes generated from 331 samples with parasite clearance rates, adjusting for population structure, patient age, parasitemia at diagnosis, and study site. Statistically significant associations were observed between parasite clearance rate and SNPs on multiple chromosomes, with large clusters of significant SNPs on chromosomes 6, 9, 10, 11, 13, and 14. These results suggest that the phenotype of parasite clearance rate following treatment with artemisinins has a multigenic basis. Potential candidate genes within identified regions will be discussed.

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USING SCANS FOR SELECTION TO IDENTIFY GENES THAT UNDERLIE ARTEMISININ RESISTANCE

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Strong selection with antimalarials has generated several textbook examples of selective sweeps, in which resistance genes have spread through parasite populations purging genetic variation, while generating high frequency long haplotypes and elevated levels of geographical differentiation. Such molecular signatures of selection are readily detected from genomic data. We used a simple two-phase strategy to identify novel drug resistance loci that minimizes the multiple-testing penalties that result from brute force genome wide association approaches. First, candidate regions were identified by examining signatures of selection in genome-wide SNP data. Second, these candidate regions were directly screened for association with resistance phenotypes in a large sample of parasites. We applied this approach to emerging artemisinin resistance in SE Asia. Initially we compared the genomes of 91 parasites from W. Cambodia (slow clearance rate (CR)), Thailand (intermediate CR) and Laos (rapid CR) by genotyping 45K SNPs on a Nimblegen microarray. This identified 33 regions within the top 1% of genome wide values for FST and XP-EHH, statistics that measure differentiation between populations in SNP frequency and haplotype structure respectively. Encouragingly, these regions contained 4/5 known resistance loci (Pfcrt, dhfr, dhps and GTP cyclohydrolase I), confirming that the regions identified are enriched for loci involved in drug resistance. We are currently screening 96 SNPs within these 33 regions for direct association with CR in an independent sample of 768 unique single-clone parasites from the Thailand-Burma border for which CR has been determined using 6-hourly measurement of parasitemia following treatment with artesunate. Targetted genotyping of small numbers of SNPs reduces the multiple testing problem, allowing statistically powerful screens with relatively low sample size. We evaluate the success of this approach and report the loci identified.

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ARREST OF HEMOGLOBIN DIGESTION RENDERS MALARIA PARASITES INSENSITIVE TO ARTEMISININ

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Artemisinin-based regimens are the frontline treatment for resistant *Plasmodium falciparum* malaria. Artemisinin (Art) is thought to be activated by opening of its endoperoxide ring leading to potent cytotoxic radicals which may damage essential macromolecules in the parasite. However, the nature and origin of the activator and the molecular basis of the Art activity are matter of debate. To better characterize the antimalarial action of Art, we used a flow cytometry-based assay to analyze the effects of drugs on parasite growth and parasitemia. Parasite cultures were pulsed (4h) with different concentrations of Art or its derivatives and monitored using SYTO 61, a nuclear dye, through two parasite life cycles (26 and 56 h respectively). Hemoglobin uptake and parasite viability were simultaneously assessed employing parasites that had invaded resealed RBCs containing fluorescein-dextran. We found that drug treatment slows parasite growth and inhibits uptake of hemoglobin, even at sub-lethal concentrations. We also examined whether inhibition of hemoglobin degradation compromises artemisinin activity. The cysteine proteases, falcipain-2 and falcipain-3, play major roles in hemoglobin degradation by intraerythrocytic parasites. Falcipains cleave hemoglobin releasing heme. Inhibition of hemoglobinase activity with the cysteine protease (falcipain) inhibitors, E 64 and ALLN, a calpain inhibitor, significantly decreases artemisinin sensitivity. This finding was substantiated when the falcipain-2 deletion mutant 3D7_ΔFP2 was substantially protected against an artemisinin pulse at the mid-trophozoite stage showing a ~6-fold increase in the IC_{50} value. A fluorescent oxidation reporter, DCF, was used to assess oxidative stress in drug-treated parasites. Art treatment increases the DCF signal, however pre-treatment with the protease inhibitor ALLN completely abrogated the endoperoxide-induced increase in DCF signal. Arrest of hemoglobin digestion by early stage parasites provides a mechanism for surviving short-term artemisinin exposure. Our data strongly suggest that a hemoglobin degradation product (heme or ferrous iron) is needed for the potent antimalarial activity of artemisinin. These insights are important to the design and use of new antimalarials and to the interpretation of emerging data on artemisinin resistance.

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CAPACITY OF *AEDES AEGYPTI* TO VECTOR DENGUE UNDER SMALL AND LARGE DIURNAL TEMPERATURE FLUCTUATIONS

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Few vector competence studies have considered the effect of real-world daily temperature fluctuations on the transmission of vector-borne pathogens. Many investigators have examined the effects of constant temperatures on vector competence, but these are insufficient for understanding how a mosquito's role in transmission is influenced by the variable conditions found in nature. We are investigating the effect of diurnal temperature fluctuations on dengue virus infection and dissemination, and life history traits in the primary mosquito vector *Aedes aegypti*. We use environmentally relevant temperature profiles from Thailand with small (~8°C) and large (~19°C) diurnal temperature ranges around a common mean of 26°C relative to a control temperature regime that is constant 26°C. We are testing the hypothesis that the magnitude of diurnal temperature fluctuations drives seasonal changes in dengue transmission dynamics by influencing components of vectorial capacity; i.e., vector competence and longevity. Our assessment of life

history traits under fluctuating temperature regimes allows us to explore and better understand *Ae. aegypti* population dynamics when they are exposed to various magnitudes of diurnal temperature ranges. Analysis to date shows that large fluctuations in temperature reduce larval survival and slow egg to adult development time by more than 20 hours (X^2 = 71.66, p < 0.01) compared to mosquitoes reared under a constant 26°C. Large daily temperature fluctuations negatively influence the mean number of eggs laid per female (~220 eggs *vs.* 160; $F_{2, 65}$ = 5.44, p < 0.01), eggs per gonotrophic cycle (65 *vs.* 52; $F_{2, 68}$ = 6.01, p < 0.01) and gonotrophic cycles per female (3.3 *vs.* 3.1; $F_{2, 65}$ = 6.93, p < 0.01) after 14 days of feeding on human blood. Results support the notion that fluctuating temperatures affect vectorial capacity and seasonal changes in dengue transmission in Thailand. An improved understanding of realistic temperature fluctuations on *Ae. aegypti*-dengue virus interactions will lead to more effective dengue surveillance and intervention.

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PREDICTION OF DENGUE INCIDENCE USING SEARCH QUERY SURVEILLANCE

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The use of internet search data has been demonstrated to be effective at predicting influenza incidence. This approach may be more successful for dengue which has large variation in annual incidence and a more distinctive clinical presentation and mode of transmission. We gathered freely-available dengue incidence data from Singapore (weekly incidence, 2004-2011) and Bangkok (monthly incidence, 2004-2011). Internet search data for the same period were downloaded from Google Insights for Search. Search terms were chosen to reflect three categories of dengue-related search: nomenclature, signs/symptoms, and treatment. We compared three models to predict incidence: a step-down linear regression, generalized boosted regression, and negative binomial regression. Logistic regression and Support Vector Machine (SVM) models were used to predict a binary outcome defined by whether dengue incidence exceeded a chosen threshold. Incidence prediction models were assessed using r^2 and Pearson correlation between predicted and observed dengue incidence. Logistic and SVM model performance was assessed by the area under the receiver operating characteristic curve. Models were validated using multiple cross-validation techniques. The linear model selected by AIC step-down was found to be superior to other models considered. In Bangkok, the model has an r^2 = 0.943, and a correlation of 0.869 between fitted and observed. In Singapore, the model has an r^2 = 0.948, and a correlation of 0.931. In both Singapore and Bangkok, SVM models outperformed logistic regression in predicting periods of high incidence. The AUC for the SVM models using the 75th percentile cutoff is 0.906 in Singapore and 0.960 in Bangkok. In conclusion, internet search terms predict incidence and periods of large incidence of dengue with high accuracy and may prove useful in areas with underdeveloped surveillance systems. The methods presented here use freely available data and analysis tools and can be readily adapted to other settings.

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ESTIMATES OF THE DEGREE AND LENGTH OF CROSS-PROTECTION BETWEEN DENGUE SEROTYPES FROM TIME SERIES MODELS

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Over sixty years ago, Albert Sabin provided evidence of short-term cross protection between dengue serotypes from human experimental data. Since that time, few other studies have addressed this phenomenon. Several studies have suggested that the inclusion of short-term cross-protection between dengue serotypes is critical to creating transmission models that show behavioral similar to empirical data. However, none of these models have explicitly estimated the duration and strength of cross-protection. Here, we present evidence of short-term cross protection between dengue serotypes using data from a large tertiary hospital in Bangkok (Queen Sirikit National Institute of Children's Health). Our data describes the serotype-specific incidence of hospital-attended dengue from 1973 to 2010. We use a discrete time transmission model to estimate the transmissibility of each dengue serotype as well as the duration and strength of protection provided individuals who have recently been infected with particular dengue serotypes against heterotypic infection and illness. We find evidence of 50-75% protection lasting for 2-3 years. These results are robust to several model formulations. We discuss the dynamic implications of our work and the possible impact on vaccine trials and future vaccine programs.

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THE CHANGING EPIDEMIOLOGY OF DENGUE IN THAILAND: INSIGHTS FROM SEROLOGICAL STUDIES CONDUCTED IN THE SAME LOCATION, 30 YEARS APART

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Dengue fever (DF) and dengue hemorrhagic fever (DHF) have traditionally caused substantial morbidity and mortality among children <15 y of age in Southeast Asia. However, over the past years a significant increase in the mean age of cases has been reported, in spite of a constant number of incident DHF cases. The reasons for this shift are not fully understood. Using data from two age stratified serological surveys conducted among school children in 1980 (n=1009) and 2010 (n=1811) in Rayong, Thailand, we estimated serotype specific forces of infection (FOI), a measure of transmission intensity, and the basic reproductive number (R0) of dengue for the periods 1969-1980 and 1992-2010, respecively. Past exposure to dengue was determined using single dilution neutralization test (SDNT), an assay that differentiates between primary and secondary infection and is serotype specific for those subjects that have been exposed to a single dengue serotype. We found a significant decrease in the FOI, accompanied by a smaller decrease in R0 and critical vaccination fraction. A similar pattern was observed for all four dengue serotypes and when analyzing the data at smaller spatial scales. This is consistent with the idea that the observed age shift might be a consequence of the demographic transitions that Thailand and other SE Asian countries have been undergoing and not of a true decrease in transmission by the vector. We present the evidence for this and other hypotheses. These findings have important implications in the design and implementation of dengue control interventions.

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EXPANSION FACTORS: A KEY STEP IN ESTIMATING DENGUE BURDEN AND COSTS IN SOUTHEAST ASIA

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Dengue is the most common arthropod-borne disease of humans and, unlike most infectious diseases, its incidence is increase. Dengue represents a substantial burden in many tropical and sub-tropical regions, with South-East Asia having the highest dengue incidence. Decisions about the implementation of existing and new technologies require information about the disease burden. The most difficult step is estimating disease incidence accurately. The total incidence of symptomatic dengue illness is not fully captured by surveillance systems, which usually under report the number of cases. The total number of dengue cases may be estimated using an expansion factor (EF), the number by which reported cases need to be multiplied to obtain the true number. This study is using EFs to project dengue cases and costs throughout SE Asia by year. We conducted a systematic literature review (1995-2011) and identified 9 published papers reporting original, empirically derived EFs or the necessary data from SE-Asia. EFs are based on: total cases/hospitalized case (H), total cases/diagnosed dengue (D), or total cases/reported case (R). Two EFs are based on a study of children cohorts in Kamphaeng Phet, Thailand (4.8H,3.4H), another looks at children cohorts in Kamphaeng Phet and Ratchaburi (8.4R); two are from Bandung, Indonesia--based on dengue hemorrhagic fever surveillance in 4 major hospitals (4.3R) and surveillance of a cohort of adults (2.3H); two are from Viet Nam--children cohorts in Long Xuxen (5.8R) and patients at community health posts at Binh Thuan (6.2D); and finally, two are from active community-based surveillance of children in Kampong Cham, Cambodia (7H, 9.3R). Despite SE Asia's long standing surveillance systems, these studies documented considerable underreporting. EFs in SE Asia varied by dengue definition, age group, urban/rural, geography, etc., and ranged from 2.3H (adults, Vietnam) to 9.3R (children, Cambodia). In conclusion, studies that make no adjustment for underreporting would seriously understate the burden and cost of dengue in SE Asia.

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DENGUE DYNAMICS IN THE 2009 OUTBREAK IN ORAN, ARGENTINA: IMPLICATIONS FOR MONITORING AND CONTROL

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After more than eighty years Dengue reemerged in Argentina in 1997. Since then, the largest epidemic in terms of geographical extent, magnitude and mortality, was recorded in 2009. In this work we analyzed the space-time dynamic of the Dengue epidemic in Oran, Salta province, one of the main epicenters of the outbreak. We also studied its correlation with demographic, socioeconomic and entomological factors. The city of San Ramon de la Nueva Oran is located in one of the main route of introduction of Dengue to northwest Argentina. Cases were diagnosed by UM-ELISA and MAC-ELISA (IgM) between January and June 2009. Demographic and socioeconomic data by neighborhood were obtained from the Provincial Statistics Direction. Diagnosis date and place of residence of patients were entered on a Geographic Information System using vector format cartography and Gauss Kruger coordinates into ArcGIS 9.3 software. We applied a space-time scan statistic under Poisson model considering city neighborhoods as the spatial unit and day as the temporal unit. Spearman correlation was used to study associations between socioeconomic variables and Dengue incidence. Larval house (LH) and Breteau (B) indices of *Aedes aegypti* space-time distribution was smoothed by kernel density. The epidemic started from an imported case from Bolivia which generated two seminal clusters on February 26 and 27, in the northeast and the south of the city with risk ratios of 32.9 and 36.4 respectively ($p < 0.001$). Following cases spread around the city without significant space-temporal clustering. No statistically significant association between socioeconomic variables and dengue incidence by neighborhood was found but positive correlation between population size and the number of cases ($p<0.05$) were detected. Larval indices show maximum values for the month of January (B=21.96; HL=8.39) with a gradual decrease until June. The lack of correlation between socioeconomic variables and incidence show that in this case socioeconomic conditions are not risk factors for Dengue transmission.

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RE-EMERGENCE OF DENGUE VIRUS SEROTYPE 3 IN PUERTO RICO: CHARACTERIZATION OF A DISTINCTIVE EXPANSION PATTERN

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Dengue virus (DENV) exists as four serotypes, each containing multiple genetically distinct genotypes. Colonization drives divergence of distinct lineages, generally grouped by region of isolation. Following a 21 year absence of DENV-3, the Indian Subcontinent strain emerged in Puerto Rico in 1998. The subsequent, progressive expansion of DENV-3 in Puerto Rico correlates with the displacement of the other serotypes and is representative of what occurred in the rest of the Americas. We sequenced complete genomes of 92 DENV-3 isolates obtained from clinical cases to characterize genetic diversity, phylogeography, and molecular evolution throughout 10 years of continued sampling in Puerto Rico (1998-2007). Genetic lineages were then associated with temporal and geographical data. This analysis shows that five distinct lineages emerged almost simultaneously and evolved independently. Two of these lineages are associated to strains from the Caribbean basin and were transmitted on the island for short periods. The other three lineages are formed of autochthonous virus of foreign origin, of which two successfully accomplished long-term expansions. Temporal clustering associated to specific geographical regions was found within these three autochthonous lineages. We found evidence of sustained microevolution within the clusters and fostering of fast virus migration from these clusters to the rest of the island. These local lineages experienced a steep increase in genetic diversity during the first 5 years of expansion to then stabilize during the years of full dominance of this serotype. This is the first extensive study of DENV-3 emergence and evolution in the region. Our findings unveil a high genetic diversity and co-transmission of DENV lineages coupled with a complex dissemination pattern that is different to the evolution of every other serotype on the island. Research to further define the biological and epidemiological determinants of these transmission patterns may aid our efforts to prevent the spread and re-emergence of dengue in endemic areas.

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IMMUNOGENICITY AND EFFICACY OF A SAND FLY-BASED *LEISHMANIA* TRANSMISSION-BLOCKING VACCINE FOR CANINES

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Leishmania infantum (= *chagasi*) is the causative agent of zoonotic visceral leishmaniasis; a fatal parasitic disease if left untreated. *Leishmania*

parasites undergo two developmental cycles and one of these cycles takes place within the midgut of a sand fly. The molecular interaction between the sand fly midgut and *Leishmania* parasites is poorly understood. By performing transcriptomic analyses we identified several molecules that potentially interact with the parasites in the midgut of *Lutzomyia longipalpis*, the natural vector of *L. infantum* in Latin America. Among those, we sought to select molecules that could interfere with parasite development inside the sand fly midgut and ultimately prevent transmission of the parasite to a mammalian host. Molecules abundantly transcribed during blood meal digestion were selected as ideal targets for transmission-blocking vaccines and antibodies were generated against these molecules via DNA vaccination. Sand flies were infected by an artificial blood meal containing *L. infantum* promastigotes and naive or target-immunized mouse sera. One particular *L. longipalpis* midgut molecule, LuloPer1, generated antibody that reduced the parasite load within the sand fly and resulted in a significant decrease in the mean number of parasites present during the infectious stage of the sand fly by 71%. Additionally, feeding anti-LuloPer1 antibody decreased survival by 27% six days after the blood meal. Canines are the principal reservoir for *L. infantum* in Latin America; thus, we are currently vaccinating dogs with LuloPer1 to assess its immunogenicity and efficacy in canines. For this purpose, recombinant LuloPer1 was expressed in a eukaryotic system and purified. Encouragingly, preliminary data show that vaccination of uninfected and infected asymptomatic dogs elicits a specific antibody response to LuloPer1. The identification of a molecule that can reduce or abrogate the transmission of *L. infantum* by *L. longipalpis* has implications for public health, blocking the spread of *Leishmania* between dogs and from dogs to humans.

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CAUDAL REGULATES THE TRIPARTITE INTERACTIONS BETWEEN THE INNATE IMMUNE SYSTEM, THE MICROBIOTA AND THE MALARIA PARASITE *PLASMODIUM FALCIPARUM*

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Anopheles gambiae, the major vector for the human malaria parasite *Plasmodium falciparum* in sub-Saharan Africa, uses its innate immune system to defend against *Plasmodium,* mainly via the Toll and Imd (Immune Deficiency) signaling pathways. Interestingly, these immune pathways are also activated by the microbiota present in the mosquito midgut, which is the primary site for *Plasmodium* invasion and development (Dong *et al.,* 2006 and 2009).*Caudal* was first identified in *Drosophila* as a developmental transcription factor as well as a negative regulator of the Imd pathway -mediated activation of the Relish transcription factor, as reported previously. We have shown through RNAi-based silencing assays that depletion of the *An. gambiae Caudal* results in a significant reduction of the midgut microbiota as well as a change of its species composition. Additionally, antimicrobial peptides (AMPs) are significantly upregulated upon silencing of *Caudal*. We also present studies on *Caudal*'s role in regulating the midgut microbial load and composition in field-derived *Anopheles arabiensis* mosquitoes, a key vector of malaria in southern Zambia. In these studies, the silencing of *Caudal*-silenced mosquitoes had approximately two-fold less bacteria compared to wildtype mosquitoes. Interestingly, *Caudal* is also a highly potent regulator of vector competence for *P. falciparum* while its implication in the defense against the rodent parasite *P. berghei* was weak. Our previous studies have also shown that the Imd pathway more efficiently defends against *P. falciparum* than *P. berghei* as reported previously. These findings suggest that the *An. gambiae Caudal* can influence the finely tuned tripartite interactions between the innate immune system, the midgut microbiota, and the *Plasmodium* parasite as a factor of the Imd pathway. We are currently conducting comprehensive whole-genome microarray gene expression studies to better understand *Caudal's* relationship to the Imd and Toll pathways and to identify potent anti-*Plasmodium* effectors that are transcriptionally controlled by this immune response regulator.

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REGULATION OF ANTI-*PLASMODIUM* IMMUNITY BY THE TRANSCRIPTION FACTOR LL3 IN *ANOPHELES GAMBIAE*

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Mosquitoes of the genus *Anopheles* serve as the obligate vectors of the malaria parasite *Plasmodium*. During its development within the mosquito host, several factors and developmental bottlenecks limit parasite success, including the mosquito innate immune response. Recently, we have identified an ortholog of the vertebrate transcription factor LITAF in the *Anopheles gambiae* genome named LITAF-like 3 (LL3). In response to midgut invasion by mouse and human malaria parasites, LL3 is up-regulated in the mosquito midgut epithelium. Oocyst numbers are significantly increased upon the RNAi-mediated knockdown of LL3, implicating its involvement in limiting *Plasmodium* parasite success. Upon dsRNA knockdown of LL3, the mRNA abundance of SRPN6 (an inhibitor of *Plasmodium* development) is significantly decreased in the mosquito midgut. In addition, electrophoretic mobility shift assays demonstrate that recombinant LL3 protein binds to regulatory regions within the SRPN6 promoter, suggesting that LL3 directly regulates SRPN6. Further identification of the downstream targets of LL3 was conducted by microarray analysis, resulting in the differential expression of 747 probes. Current experiments aim to identify the function of a subset of these genes affected by the knockdown of LL3 and to elucidate the mechanism by which LL3 confers anti-*Plasmodium* defenses in the mosquito midgut.

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DISSECTING THE PIRNA PATHWAY'S ROLE IN TRANSPOSON CONTROL IN THE IMPORTANT DISEASE VECTOR *AEDES AEGYPTI*

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The most recent class of small RNAs to be discovered is the Piwi interacting piRNA. PiRNAs were first discovered and reported in mice. PiRNA biogenesis is distinct from the siRNA and microRNA biogenesis in that it is involved in a dicer-independent pathway. PiRNAs are 24-30nt small RNAs that bind Piwi proteins. The Piwi class proteins were first discovered over ten years ago in drosophila mutants and are members of the Argonaute clade. Currently piRNAs are believed to be involved in transposon and endogenous retrovirus control in both the germline and somatic cells, as reported previously. The biogenesis pathway of piRNAs is the least understood of the current three small RNA pathways. The ping-pong model of piRNA production suggests that Argonaute proteins work together in an autoamplification loop and is currently the strongest hypothesis being considered, as reported previously. The role piRNAs play in transposon control is only beginning to be understood. The important proteins involved in the pathway have been identified but the details in how they function together to create a targeted response are still not fully worked out. Our research investigates the function, expression and targets of the piRNAs associated with the PIWI protein in *Aedes aegypti*. To date no investigations into the piRNA pathway have been performed in mosquito systems. Here we aim to determine the tissues and developmental stages in which the putative Piwi proteins are expressed in the medically significant *Aedes aegypti*. We have characterized the piRNAs that are bound to the Piwi protein via co-immunoprecipitation *in vivo*. The isolated piRNAs were analyzed bioinformatically and mapped to the *Aedes aegypti* genome to determine the targets of these small RNAs. The information provided in this study provides valuable insight into how

the Piwi proteins are functioning in mosquitoes. This knowledge may in turn help us understand transposon control thus enabling us to develop techniques to circumvent transposon silencing and boost transformation efficiency of mosquitoes

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BRUMMER LIPASE AND ADIPOKINETIC HORMONE LIPOLYTIC PATHWAYS ARE REQUIRED TO GENERATE LIPIDS NECESSARY FOR TSETSE MILK PRODUCTION

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Tsetse females reproduce by obligate viviparity, generating a single larva per gonotrophic cycle, and larval nourishment is derived from milk secretions provided by the mother. Optimum nutrition during pregnancy requires mobilization of large quantities of lipids for production of milk during intrauterine larval development. We investigated the role two lipolytic pathways (Brummer lipase (Bmm) and adipokinetic hormone (AKH)/AKH receptor pathways) on providing lipids for milk production. Two putative adipokinetic hormone (AKH) coding genes, (*akh* and *hrth*), *akhr* and *bmm* were identified from *Glossina morsitans morsitans*. Expression of *akh*, *hrth* and *akhr* increase at the end of oogenesis during the first gonotrophic cycle, then decrease and stabilize throughout larval development. Levels of *bmm* increase during the early progeny development and decline at parturition. Knockdown of *bmm* (*bmm*$^-$) and *akhr* (*akhr*) was accomplished utilizing siRNA injection. Suppression of one lipolytic pathway results in an increase in transcription of the other. Starvation-based experiments on females revealed thay *bmm*- and *akhr*- flies had prolonged survival. Simultaneous reduction of both genes extends survival by an additional 20%. Flies with the *akhr*/*bmm*$^-$ knockdown have higher lipid contents upon death, indicating the inability to completely utilize stored lipid reserves. Oocyte development was impaired by knockdown of *bmm* and impairment was even more dramatic in *akhr*/*bmm*$^-$ flies. Flies with *akhr*$^-$ and *bmm*$^-$ knockdown have 20 and 50% reduction in fecundity, respectively, and *akhr*$^-$/*bmm*$^-$ flies have an 80% decrease. Omission of one bloodmeal (short period of starvation) for *akhr*/*bmm*$^-$ flies leads to almost complete suppression of reproduction. The reduced level of fecundity is likely due to the inability of tsetse to utilize lipid reserves as the *akhr*/*bmm*$^-$ phenotype leads to increased lipid accumulation and retention, particularly during pregnancy. These studies show that Bmm and AKH/AKHR pathways are critical to producing lipids necessary for milk production during tsetse fly pregnancy.

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MEASUREMENT OF THE SCUTAL INDEX AND DISPARATE ACQUISITION OF *BORRELIA BURGDORFERI* GENOTYPES IN LARVAL *IXODES SCAPULARIS* TICKS

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Borrelia burgdorferi, the causative agent of Lyme borreliosis in the United States, is comprised of many genotypes, which are associated with varying degrees of dissemination in host species. These genotypic strains are known to infect *Ixodes scapularis*, the primary vector, disparately. Field studies often examine larval-stage ticks removed during their host feeding periods as an indicator of infection in the host. Characterization of these various genotypes during larval feeding has yet to be done, adding a source of uncertainty in determining host infection. In addition, feeding duration needs to be correlated with pathogen acquisition in order to associate various time points during feeding with the probability of a tick becoming infected. Here we quantitatively describe how *I. scapularis* larvae obtain two different genotypes of *B. burgdorferi* during 12 hour intervals over the course of the feeding period. Uninfected larvae were placed on *Peromyscus leucopus* previously infected with either a highly-disseminating or low-disseminating strain, Bb206 or Bb348. Larvae were removed every 12 hours for 72 hours. We measured the scutal index as an indicator of feeding duration and ticks were assayed using a quantitative PCR protocol. The scutal index revealed a direct relationship between scutal index measurement and length of feeding. Results contrast significantly between the two strains of Borrelia. Ticks infected with Bb206 had increased quantities of Borrelia per tick and produced more infected ticks at each time point of feeding as compared to Bb348, but was only significant at hours 36, 42, and 72. Ticks that fed on Bb206-infected mice acquired infection during the first 12 hours of feeding, whereas ticks feeding on Bb348- infected mice required more time to become infected. In summary, our findings further describe the intricate infection characteristics of *B. burgdorferi* and will enable researches to make better estimations of infection prevalence in mammals based on the infection status of removed larval ticks.

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RHODNIUS PROLIXUS GENOMICS AND TRANSCRIPTOMICS: IDENTIFICATION AND ANNOTATION OF GENES LINKED TO THE Y CHROMOSOME AND GENES RELATED TO SEX DETERMINATION

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Due to the abundance of repetitive DNA, the sequencing and assembling of genomic heterochromatic regions is problematic. The Y chromosome is heterochromatic in most species, hindering the identification of Y-linked sequences in many genome projects. Our laboratory has developed a variety of computational and experimental methods to overcome such difficulties. These methods have allowed a series of comparative studies on the gene content of the *Drosophila* Y chromosome and we have shown recently, that the *Drosophila* Y chromosome may have a non-canonical evolutionary history. Therefore, the study of the Y chromosome of other insects may reveal new aspects of this chromosome, helping to better understand male fertility and sex differentiation systems in other arthropods. The kissing bug *Rhodnius prolixus* is the vector of the parasite *Trypanosoma cruzi* (the etiological agent of Chagas' Disease, an important neglected disease) and its genome project is in progress. By using the expertise obtained during the 12 *Drosophila* genome project, we have proposed to identify and annotate the genes linked to the Y chromosome of *R. prolixus*. We have already identified 3.8 Mbp of Y-linked sequences. We are also using transcriptome information to find genes of interest. The genomic and transcriptomic analysis revealed hundreds of potential Y-linked genes, and we are working on the full annotation of 20 of these genes. We also uncovered most of the genes related to sex determination in arthropods (e.g. *sex lethal, doublesex* and *fruitless*), suggesting that Hemiptera insects may have a sex determination system similar to Diptera. Our preliminary results have revealed that separate sequencing of male and female genomic DNA may turn out to be a powerful method for finding sequences of the Y chromosome. The combination of methods proposed here may help us to better understand the mechanisms involved not only in the origin and evolution of sex chromosomes, but also the mechanisms involved in sex determination and male fertility of non-dipteran arthropod vectors.

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ENTAMOEBA MOSHOKOVSKII IS PATHOGENIC AND CAUSES INTESTINAL SYMPTOMS

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Entamoeba moshkovskii is prevalent in the developing countries and morphologically indistinguishable from pathogenic *E. histolytica* and non-pathogenic *E. dispar*. As *E. moshkovskii* has been considered to be a non-pathogenic or a free living amoeba, there are few studies to elucidate the pathogenicity of it. Thus, we carried out the study to clarify the pathogenicity of *E. moshkovskii*. To examine the pathogenicity of *E. moshkovskii*, animal model of intestinal amoebiasis was utilized. Trophozoites of *E. moshkovskii, E. histolytica* or *E. dispar* were challenged to mice and infection rate was examined. In successfully infected mice, intestinal symptoms, weight loss and time course of infection were observed. Then the prevalence of each *Entamoeba* spp. in diarrheal episode was examined in Bangladesh and several cases that were positive only for *E. moshkovskii* but negative for other conceivable diarrhea-causative microbes were found. *E. moshkovskii* settled in CBA/J, C3H/HeN and C3H/HeJ mice, but not in C57BL/6J and BALB/c mice similar to pathogenic *E. histolytica*, while non-pathogenic *E. dispar* could not establish the infection in mice. *E. moshkovskii* induced intestinal symptoms and weight loss in mice. In Mirpur, Dhaka, Bangladesh, *E. moshkovskii* was identified in 42 diarrheal episodes (2.95%) out of 1426 diarrheal episodes in 385 children, while *E. histolytica* was in 66 (4.63%) and *E. dispar* was in 5 (0.35%), indicating the association of diarrhea with pathogenic *E. histolytica* and *E. moshkovskii*. Six episodes were confirmed to be solely associated with *E. moshkovskii*, without detection of any conceivable diarrhea-causative microbes. *E. moshkovskii* was found to be pathogenic to mice and to well associate with diarrheal episode in Bangladeshi children. Therefore it is important to re-estimate the pathogenicity of *E. moshkovskii*.

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COMMON PATHWAYS FOR THE RECEPTOR-MEDIATED INGESTION OF *ESCHERICHIA COLI* BACTERIA AND LDL CHOLESTEROL BY *ENTAMOEBA HISTOLYTICA* REGULATED BY TRANSMEMBRANE KINASE (TMK) 39

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The single-celled parasite *Entamoeba histolytica* is an enteric pathogen that ingests bacteria and host cells as it causes intestinal disease. The ligand/receptor interactions that allow *E. histolytica* to phagocytose such extracellular targets are not well understood. We hypothesized that trophozoites of this parasite might accomplish ingestion through the utilization of scavenger receptor-like mechanisms. Here we show that acetylated and oxidized forms of LDL cholesterol (AcLDL and OxLDL) were phagocytosed by ameba via receptor-mediated mechanisms, whereas pinocytosis of dextran was not. AcLDL competitively inhibited the ingestion of *E. coli* bacteria, but not erythrocytes and Jurkat T lymphocytes, by 31% (SE±1.34) (P<.005), suggesting a common phagocytic pathway. Inducible expression of a truncated dominant-negative version of *E. histolytica* transmembrane kinase 39 (TMK39) inhibited ingestion of *E. coli* by 55%±2.99 (P<.005). We concluded that two pathogenic processes, ingestion of AcLDL and of *E. coli,* shared common pathways and regulation.

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EXPRESSION AND PURIFICATION OF RECOMBINANT LECA PEPTIDE AS A CANDIDATE FOR AN AMEBIC COLITIS VACCINE

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Entamoeba histolytica causes amebic colitis and liver abscess. It is a major enteric pathogen in developing countries and for travelers returning from endemic areas. The interaction of *E. histolytica* with the intestine occurs through the binding of the trophozoite stage via a Gal/GalNAc lectin comprised of disulfide linked heavy (180 kDa) and light chains (35 kDa) and noncovalent binding with an intermediate subunit (150 kDa). Our efforts to develop a vaccine against this pathogen have focused on an internal 578 amino acid peptide, LecA, within the cysteine-rich region of the heavy chain subunit because (i) it is a major target of the cell mediated and humoral immune response of immune individuals and (ii) vaccination with LecA provides protection in animal models. We developed a process for obtaining >95% homogeneous LecA. The lecA gene sequence was optimized for expression in Escherichia coli, and LecA was expressed in host strain HMS174 in the vector pJ express 401 containing a Kanr gene and a T5 promoter. More than 80% of the peptide was expressed in inclusion bodies (IB). The process consisted of three stages: (i) cell lysis, collection and washing of IB; (ii) solubilization and refolding of LecA; and (iii) Superdex 200 gel filtration. SDS-PAGE demonstrated a major peptide (70 kDa) identified as LecA by N-terminal sequencing and tryptic digest analysis. The LecA band and minor bands reacted with monoclonal antibodies against LecA. LecA had an apparent MW of 70,000 by SDS-PAGE compared to 158,000 by gel filtration. Endotoxin levels were 0.20 EU/µg, and DNA and RNA levels were <200 ng/mg and <20 ng/mg, respectively. The purified peptide exhibited higher residual immunoreactivity than his-tagged LecA, which is protective in a murine model of amebic colitis, indicating that protective epitopes were conserved. The procedure is scalable to cGMP and yields 20 mg per liter shaking flask culture. Our procedure yields sufficient amounts of highly purified LecA for future studies on stability, immunogenicity, and protection studies.

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VALIDATING MULTIPLEX REAL-TIME PCR FOR THE DETECTION OF INTESTINAL PARASITES IN THE CLINICAL LABORATORY

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A molecular diagnostic strategy was evaluated for the routine diagnosis of intestinal parasites in stool specimens. The possibility of high throughput multiplex detection of stool intestinal parasites may ultimately result in rapid and sensitive testing. Unpreserved stool specimens were collected from symptomatic patients samples in Toronto, Ontario. Stool samples were analyzed on a routine basis by microscopic examination for ova and parasites. Both positive and negative stool samples were banked in a stool biorepository by preparing five aliquots of each specimen in a 1.5 ml screw capped vials and stored at -20°C for batched molecular testing. Multiplex real-time polymerase chain reaction (RT-PCR) analysis was designed to detect *Entamoeba histolytica*, *E, dispar*, *Giardia lamblia*, *Cryptosporidium* (*C. parvum / C. hominis*), *Dientamoeba fragilis, Cyclospora cayetanensis, Strongyloides stercoralis, Necator americanus*, and *Ascaris lumbricoides* using a single or two multiplex panels in 96 well plates. In a pilot run the RT-PCR was performed on 50 stool samples

which included 10 negative and 40 positive stool samples by routine microscopy. The 40 positive stool samples included *E. histolytica* (n=10), *Giardia lamblia* (n=4), *Cryptosporidium* (n=3), *Dientamoeba fragilis* (n=10), *C. cayetanensis* (n=10), *Strongyloides stercoralis* (n=2), and *Microsporidium* (n=1) by microscopy. The RT-PCR result was concordanct with all microscopy positive stool samples. In addition, RT-PCR also picked a mixed infection (*Strongyloides stercoralis* and *Isospora belli* and *Necator americanus*) in one stool sample which was exclusively positive for *S. stercoralis* by microscopy. Interestingly, the RT-PCR was positive in 2 out of 10 stool samples negative by microscopy: *Schistosoma mansoni* and *Enterocytozoon bieneusi*. In summary, we have developed a multiplex RT-PCR panel to simultaneously detect eleven stool intestinal parasites. The pilot study conducted demonstrated excellent concordance with microscopy. Furthermore, several specimens negative by microscopy were positive by RT-PCR for significant stool pathogens. Superior sensitivity and throughput of multiplex RT-PCR methods in comparison to routine microscopy suggest that increased detection rates and improved turn around times may be possible for certain stool parasites.

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EFFECTS OF HABITAT DISTURBANCE ON HOST COMMUNITY STRUCTURE AND PATHOGEN PREVALANCE

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Infectious diseases are strong forces acting on natural populations and considered an important component in the structure of ecosystems. Disease dynamics can rarely be explained by examining one component of a complex natural system. Habitat quality, host community, host health status, and pathogen interactions are interconnected in complex and dynamic ways. To understand disease dynamics in natural settings, the complex interplay among habitat disturbance, host community structure, and pathogen interactions with both the host and any co-infecting pathogen must be understood. This study examines the small mammal community in western Uganda and the pathogens infecting these animals. We collected 348 small mammals from habitats experiencing varied levels of habitat disturbance in and around Kibale National Park, western Uganda. Each animal was identified to species level and screened for the presence of ectoparasites, protozoans, and viruses. Our results suggest that increases in habitat disturbance are linked, to a point, with an increase in diversity of small mammals and prevalence of their pathogens. We aim to more accurately capture the true complexity and dynamic nature of pathogen-host ecology by investigating the interactions between multiple sympatric pathogen-host systems in areas experiencing varied levels of disturbance.

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MONITORING TRANSMISSION OF *WUCHERERIA BANCROFTI* AMONG A SENTINEL COHORT OF PAPUA NEW GUINEAN CHILDREN USING A COMBINATION OF DIAGNOSTIC ASSAYS

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Monitoring of existing national lymphatic filariasis (LF) elimination programs is based on the WHO recommended immunochromatographic test (ICT) that detects adult *Wuchereria bancrofti* (Wb) antigen in blood. Though convenient for identifying areas eligible to start mass drug administration (MDA), the ICT shows variable efficacy following longstanding MDA programs. To evaluate whether newer serological measures of assessments of Wb infection/exposure would be more useful than the ICT test, a sentinel population of 1-10 year old children was studied to assess the best approach. Serum samples were obtained from children born in communities that had completed 5 rounds of annual MDA ten years previously. These samples were evaluated with an L3 stage-specific WB-123 LIPS assay and compared to LF monitoring tools including microscopy for microfilaria (MF), PCR, circulating antigen tests (ICT and TropBio™), and an alternative antibody test (Bm14). Among the 422 children studied, 9 were MF positive. With regard to the 9 MFpositive children, tests for antigen and antibody to BM14 were 100% concordant. One and two MF positive children were negative by WB-123 and PCR, respectively. Ability of an assay to detect suspected LF transmission (i.e., highest sensitivity) was evaluated as the percent of 413 MF negative children who tested positive in each alternative assay. Increased sensitivity over microscopy for MF was 3% for PCR, 5% and 9% for antigen (Binax and Og4C3, respectively), and 11% and 26% for antibody (Wb-123 antibody and BM14, respectively). Test positivity among MF negative children may represent pre-patent, single-worm, non-fecund infections, or non-infective exposure to Wb. Results show varying outcomes observed when targeting different parasite and host bio-markers of Wb infection. LF elimination programs must consider the strengths and limitations of diagnostic strategies in effective monitoring and evaluation of LF Elimination Programs.

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ANTIBODY TO L3 ANTIGEN (WB123) AS A MARKER OF BANCROFTIAN FILARIASIS TRANSMISSION IN THE SOUTH PACIFIC

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Antibody (Ab) to the *Wuchereria bancrofti* (Wb) infective larval (L3) antigen Wb123, detected by a Luciferase Immunopreciptation System (LIPS) assay, has been shown to be a species-specific, early marker of infection with Wb developed for its potential use as a surveillance tool following transmission interruption. To examine its usefulness in a single filarial-endemic island (population ~600) assessed at two time points with markedly different levels of transmission, Ab to Wb123 was measured in sera collected from subjects in Mauke, Cook Islands in 1975 (n=369; no previous treatment) and 1992 (n=553; 5 years after a one time island-wide treatment with diethylcarbamazine [DEC]). Between the two time points, Wb transmission had decreased dramatically as evidenced by reduced prevalence of microfilariae (31% vs 5%) and circulating Ag (CAg, 49% vs 16%). Age specific prevalence analysis showed an even more dramatic reduction in Wb123 Ab positivity from 54% (25/46) in 1975 to 8% (3/38) in 1992 in children 1-5 years ($p<0.0001$), reflecting the single-dose DEC treatment five years earlier. By 1992, Wb123 Ab prevalence in children 6-10 years had fallen from 75% (42/56) in 1975 to 42% (33/79) reflecting a lower cumulative transmission potential. In the whole population, Wb123 seropositivity decreased from 86% to 61% between 1975 and 1992. In CAg+ subjects the levels of Wb123 Ab were indistinguishable between the 2 time points (geometric mean [GM]=232,067 units [1975] vs 210,115 [1992]) but differed in those who were CAg- (GM=33432 [1975] vs 11,095 [1992]; $p<0.0001$). In paired sample analysis, individuals who were CAg+ in 1975 but became CAg- in 1992 had significantly lower Ab levels in 1992 ($p<0.0001$), with 9/40 (23%) becoming seronegative for Wb123. The clear relationship between reduction in Wb123 Ab prevalence and the reduction of transmission, seen most clearly in young children, strongly advocates for the continuing assessment and rapid development of Wb123 as a surveillance tool to detect potential transmission of bancroftian filariasis in treated endemic areas.

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USE OF ENHANCED SURVEILLANCE METHODS FOR DETECTING LOW-LEVEL PERSISTENCE OF LYMPHATIC FILARIASIS FOLLOWING CESSATION OF MASS DRUG ADMINISTRATION (MDA)

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The Sri Lanka Anti-Filariasis Campaign (AFC) provided MDA to 10 million people in 8 endemic districts between 2002 and 2006. All districts satisfied WHO criteria for filariasis (LF) elimination in 2008, but surveys showed low-level persistence of microfilaremia (Mf) in some sentinel sites. This project is using enhanced surveillance tools to detect persistent LF. Methods include community surveys to detect Mf and filarial antigenemia (ICT), school surveys for ICT and anti-filarial antibodies (Bm14 ELISA) in children 6-8 years of age, and mosquito surveys to detect filarial DNA in Culex mosquitoes collected by gravid traps (molecular xenomonitoring, MX). Criteria for LF elimination were predefined to be <0.5% for Mf (community), <2% for ICT (community), <2% for antibody in children, and <0.25% for parasite DNA in mosquitoes. The project will test two sentinel sites with populations ranging from 10,000 to 35,000 in each of the 8 formerly endemic districts. We now report results from study site A in Gampaha district and study sites B and C in Colombo district. All laboratory testing was conducted by AFC personnel. Community Mf rates were very low in all three areas (< 0.5%). Community ICT rates were 3.6, 0, and 0.5% in areas A, B, and C. ICT rates in children were 1.6, 0, 0% in areas A, B, and C, and antibody rates in children were 2% in areas B and C. Filarial DNA rates in mosquitoes in areas A, B, and C were 0.75, 0.09, and 0.5%. Thus all three study areas had low-level persistence of filariasis markers several years after suspension of MDA. Areas A and C failed to meet our criteria for LF elimination; follow-up testing will be needed in these areas. WHO recommends using child ICT survey results as the primary indicator for decisions to stop MDA and for post-MDA surveillance. However, antibody testing of children and MX appear to be more sensitive tools for detecting low-level persistence or resurgence of filariasis in communities. We recommend use of these tools for special surveys in suspected hot spots to complement systematic ICT surveys currently recommended by WHO.

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LONGITUDINAL EVALUATION OF ANTIFILARIAL SEROLOGICAL RESPONSES IN A COHORT OF HAITIAN CHILDREN

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Antifilarial antibody testing has been established as a sensitive and specific method of diagnosing lymphatic filariasis. However, the development of serological responses to specific filarial antigens and their relationship to acquisition of infection is poorly understood. In order to evaluate whether the detection of antifilarial antibodies precedes that of microfilaremia and antigenemia, we compared the antibody responses of serum samples collected between 1990 and 1999 from a cohort of 143 Haitian children followed longitudinally. Antigen status was determined using the Og4C3 ELISA and the presence of microfilaremia was detected using microscopy. Antibody responses to Wb123, a *Wuchereria bancrofti* L3 antigen, were measured using a Luciferase Immunoprecipitation System (LIPS) assay. Antibody responses to Bm14 and Bm33, *Brugia malayi* antigens, and to a major surface protein (WSP) from *Wolbachia* were analyzed using a multiplex bead assay. The median month of positivity (MM) to all parameters was determined. Over follow-up, 81 (57%) of the children became Ag+ (MM= 48), and 43 (30%) developed microfilaremia (MM= 70). Detectable antibody responses to Bm14 (MM=42), Bm33 (MM=33), Wb123 (MM=45), and WSP (MM=58) developed in 95%, 99%, 90%, and 22% of children, respectively. Peak incidence of antibody was 3, 2.5, and 2 years for Wb123, Bm14, and Bm33, respectively. Both Bm14 and Wb123 antibody prevalence were significantly greater (P<0.05) among children who became Ag+ than among Ag- children. Antifilarial antibody responses can serve as an important epidemiological indicator in a sentinel population of young children. Understanding the timing of the development of antibody responses will help to establish a framework for using antibody testing for surveillance of lymphatic filariasis in the effort to eliminate the disease.

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ARE FIVE ANNUAL ROUNDS OF MASS DRUG ADMINISTRATION (MDA) ENOUGH TO ELIMINATE LYMPHATIC FILARIASIS (LF) IN EGYPT?

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Egypt (with an estimated 2.7 million population at risk of LF) initiated one of the first national LF elimination programs (EPELF) based on mass drug administration (MDA) with DEC and albendazole in 2000. The program used villages as implementation units (IUs) and included IUs with baseline infection rates ≥1% mf. The program maintained high coverage rates (≥80% by independent surveys). MDA was discontinued in 149 IUs (92.5% of the total) that met WHO stopping criteria after 5 rounds. As these criteria have not been evaluated extensively, the present study was designed to test the WHO hypothesis that 5 MDA rounds with good coverage would eliminate LF. We selected 5 villages with the highest pre MDA mf prevalence (4 - 11%) to be our evaluation units (EU) in this study that was carried out in 2010 to find out if LF resurgence would happen in 6 years after stopping the MDA. We examined ~400 adults and all first primary school kids (age 6 - 7 years) in each village for antigenemia using ICT cards (total of 2095 adults and 1026 children). All were found to be antigen-negative. We also carried out an outdoor mosquito survey using 40 Gravid Mosquito Traps (GMT) distributed at the periphery of each village. 50 mosquitoes (the main LF vector in Egypt being Culex pipiens) were collected from each trap. Half were analysed by PCR in the Central Lab of the Egyptian Ministry of health while the other half were shipped to the US for quality control. All specimens were PCR-negative. These two sets of findings imply that no resurgence of LF occurred in Egypt in the 6 years after stopping MDA.

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LONG LASTING INSECTICIDE TREATED NETS COMPLEMENT MASS DRUG ADMINISTRATION TO ACCOMPLISH A SUSTAINED REDUCTION IN LYMPHATIC FILARIASIS TRANSMISSION

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The principle strategy of the Global Programme to Eliminate Lymphatic Filariasis (GPELF) employs annual mass drug administration (MDA) for 4-6 years to break LF transmission. Despite successes, GPELF has yet to overcome challenges of attaining high drug coverages and sustaining programs for multiple consecutive years. Also, elimination thresholds are unknown in most areas. These difficulties have led to concern that a single global strategy of elimination may not be resilient, and specifically, that lack of vector control may hinder the progress of LF elimination. There are many areas where *Anopheles* spp. transmit both LF and malaria parasites, providing the opportunity for malaria control efforts to benefit the GPELF. Currently, the most widely implemented vector control intervention in malaria campaigns is the distribution of long lasting insecticide treated nets (LLINs). However, only one study has demonstrated the positive impact of LLINs on reducing LF transmission by anophelines, and there is no data that quantifies how LLINs will complement MDA. Distribution of LLINs following MDA may increase the probability of a sustained reduction in LF transmission. To test this hypothesis, we collected mosquitoes before and after LLIN distribution (n=21,642) in an area of Papua New Guinea that had previously received MDA. Mosquitoes were examined for infection by *Wuchereria bancrofti*, and a subset was analyzed for *W. bancrofti* DNA. Shortly after LLINs were distributed, surveys indicated that 83% of study participants slept under an LLIN (n=2459). Entomological indices of transmission in 1998 (after MDA) and in 2008 (prior to LLIN) were similar. The *An. punctulatus* man biting rate was significantly reduced post LLIN. Significantly fewer mosquitoes were infected post LLIN as measured by dissections and PCR. Likewise, while 0.7% of *An. punctulatus* were infective one year prior to LLIN (n=2996), none were infective post LLIN (n=675). The inclusion of vector control in the GPELF stands to improve the long-term durability of these programs.

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IMPACT OF HUMAN MOVEMENT ON MDA COMPLIANCE AND EFFECTIVENESS IN PAPUA NEW GUINEA

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The current program to eliminate lymphatic filariasis (LF) is based on the expectation that 4 to 6 rounds of annual mass drug administration (MDA) with population coverage ≥70% will result in unsustainable transmission. However, transmission has not been halted in some regions after reportedly achieving these outcomes. Possible reasons for MDA program failure could include incomplete drug coverage, missed MDA doses due to travel, or reintroduction of infected individuals following MDA. This study analyzes four years of weekly demographic surveillance records from a field trial of annual MDA in four communities of Papua New Guinea. Weekly in- and out-migration was recorded by local reporters performing active surveillance. Of 848 individuals in four communities, 48% were absent each year for an average of one month (range:1 week-10 months). This occurred at greatest frequency during times of agricultural activity. Of the people spending time away from their village of residence, 12-41% were absent during days of drug administration. 48-62% of these individuals received ≤2 of the 5 MDAs versus 40% of the general population. Following 4 rounds of MDA, 12% of mobile individuals and 5% of permanent residents were microfilaria positive (p=0.05). Results from this study may be used to better design MDA distribution programs and improve methods to estimate program coverage.

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WASTING IN EARLY CHILDHOOD AS A RISK FACTOR FOR STUNTING

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Childhood undernutrition is a risk factor for childhood illness and death. We obtained individual-level, longitudinal anthropometry data for 1,590 children 0-2 years old from nine cohort studies and found an association between stunting at 18-24 months and wasting during the first 18 months of life. In order to further explore the longitudinal relationship between wasting and stunting, we considered stunting status at 18-24 months as a function of wasting in the age intervals of 0-5, 6-11, and 12-17 months using GEE for logistic regression and robust variances. We found that children with their first wasting measurement in the 0-5, 6-11, and 12-17 month age groups were 1.3, 2.7, and 3.1 times, respectively, more likely to be stunted at 18-24 months than children with no wasting in any time period. In addition, using a random effects model and robust variances with length-for-age z-score (LAZ) at 18-24 months as the outcome measure, children with their first wasting in the 0-5, 6-11, and 12-17 month age groups had LAZs that were 0.2, 0.6, and 0.9 z-scores lower, respectively, than those with no wasting during any of those periods. We also considered multiple periods of exposure and found that children with more recent wasting, as well as wasting in more than one 6-month age group, were more likely to be stunted at 18-24 months and to have lower LAZ scores than children who were never wasted during follow up or who were wasted only in the 0-5 month age group. Finally, since variability in weight-for-length z-scores (WLZ) due to seasonality of infection or food insecurity may result in decreased linear growth, we modeled the impact of WLZ variability during the first 18 months on LAZ at 18-24 months. Children with greater WLZ variability were more likely to be stunted and were shorter by 0.3 z-scores at the end of follow up. The results of this study indicate that children with highly variable WLZ are at particular risk for stunting and actions should be taken to decrease that variability. In addition, targeted interventions to decrease wasting in young children are likely to decrease stunting prevalence overall.

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HOW LONG DOES GROWTH IMPEDE IN CHILDREN AFTER ACUTE DIARRHEA?

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Diarrhoeal disease is the second leading cause of mortality and morbidity globally. The disease kills 1.5 million and causes 2 billion episodes of diarrhoea each year and is considered as the leading cause of malnutrition in <5 year age children. We evaluated whether moderate to severe diarrhoea has short and long term nutritional sequelae. In a case control study conducted at the rural Basse of The Gambia in Western Africa we measured the anthropometric indices of the <5 children presenting with signs of moderate to severe diarrhoea. Age and sex matched controls were enrolled from the community within a demographic surveillance system. Cases and controls were then followed up at 60-90 days and 18-24 months after the enrolment. Nutritional assessment was based on the WHO's Z scoring system. We enrolled 854 cases and 1161 controls during the two years of the study. According to the weight for age Z score (WAZ), the case children were significantly (p<0.001) more severely malnourished compared to controls [OR 3.59 (95% CI 2.65 to 4.88); OR 1.98 (95% CI 1.21 to 3.26); OR 3.54 (95% CI 2.18 to 5.78); OR 11.59 (95% CI 5.39 to 25.59); for all children, 0-11m, 12-23m and 24-59m age group respectively. A similar trend was observed on the weight for height/length Z score (WHZ). On height/length Z score (HAZ) only the 24-59 months age group of cases were significantly different to controls [OR 2.84 (95% CI 1.42 to 5.68) p 0.001]. On 60-90 day follow up a higher proportion of case children remained severely malnourished on WAZ, HAZ and WHZ scale and a significant difference was observed in WAZ and HAZ in the older age strata [WAZ-OR 2.68 (95% CI 1.17 to 6.16), p0.017; HAZ-OR 4.37 (95% CI 2.03 to 9.52), p<0.001]. Children aged <5 years fail to regain their growth compared to their peers after acute diarrhoea. A nutritional rehabilitation after an acute episode of diarrhoea is highly recommended. These cohorts need to be followed up till the preschool age to understand the long term growth faltering and their consequences in this population.

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PARASITISM IN CHILDREN AGED THREE YEARS AND UNDER: EFFECTS ON GROWTH AND VACCINE RESPONSE IN RURAL COASTAL KENYA

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Children are at high risk for helminth and protozoal infections, although estimates of parasitic burden and effects on growth are lacking in those under age 5 years. Recent evidence shows that parasitic infections may also alter vaccine response. Our objective was to document the prevalence of parasites and their effects on growth and response to childhood vaccines in young children in coastal Kenya. Stool, urine and blood samples were collected from children at 6 month intervals until age 3 years and tested for soil transmitted helminths (STH: Ascaris, Trichuris, hookworm, Strongyloides), protozoa (malaria, Giardia), and schistosomiasis. Height, weight, and head circumference (HC) were measured at each visit. Prenatal maternal helminth and protozoal infections were documented. Response to tetanus, diphtheria, hepatitis B virus, Haemophilus influenzae type B, and poliovirus vaccinations were measured by standard ELISA. McNemar's test, Student's t test on log transformed titers, and repeated measure modeling were used to analyze data. Of 545 children, 32% had parasitism and 8% had polyparasitism. Hookworm was most prevalent STH (11%), followed by Trichuris (10%), Ascaris (4%) and Strongyloides (2%). Giardia was the most prevalent protozoan (13%) followed by malaria (12%). 4% had schistosomiasis by IgG4 testing. Early childhood infection with STH, hookworm, and malaria were associated with maternal infection. Polyparasitized children were more likely to have polyparasitized mothers (p=0.01) and have poor HC growth rate (0.002). Children with hookworm (p=0.01), any STH (p=0.049) or any parasitic infection (p=0.039) had slower weight gain. Children with hookworm (p=0.04), Giardia (p=0.03), Strongyloides (p=0.001), schistosomiasis (p=0.02) or malaria (p=0.01) had slower HC growth rates. Children with hookworm, Trichuris, or Giardia had statistically lower tetanus titers than uninfected children; those with malaria or any parasitic infection had statistically lower diphtheria titers. Our results document the under recognized burden of parasitism in children aged 0-36 months in rural Kenya. Parasitic infections in this young age group have detrimental effects on weight, height, and HC growth rates and may have significant implications on child health. Certain parasitic infections in childhood, such as STH and malaria, may also be linked to decreased response to standard childhood vaccinations.

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A CLUSTER-RANDOMIZED EVALUATION OF A RESPONSIVE FEEDING AND STIMULATION INTERVENTION ON NUTRITION AND DEVELOPMENT OUTCOMES IN RURAL BANGLADESH

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Parenting education for feeding and stimulation of infants and young children is needed, particularly in South Asia, where 45% of children are malnourished and many do not achieve their learning potential. Responsive stimulation is sometimes associated with better language development. Six group sessions with mothers and young children in rural Bangladesh used demonstration and coached practice to promote responsive stimulation, feeding and hand washing. Compared with an informational control, children in the intervention group had better developmental and nutritional outcomes, including language, hand washing and mouthfuls eaten. This study was undertaken to determine whether a responsive feeding and stimulation intervention improved nutritional and developmental outcomes compared to a regular information-based parenting program. A cluster randomized trial was carried out with 302 children 8-20 mo and their mothers in rural Bangladesh, randomized by village to one of three groups. The control mothers received 12 informational sessions on health and nutrition. The intervention groups received the same 12 sessions plus 6 sessions delivered by peer educators that included modeling and coached practice in self-feeding, hand washing and verbal responsiveness with the child during play. A second intervention group in addition received 6 months of iron-fortified Sprinkles. Nutritional outcomes included weight, height, self-feeding and mouthfuls eaten. Developmental outcomes included HOME Inventory, mother-child responsive talk, and language development. Analysis of covariance compared the three groups at posttest and follow-up, covarying pretest levels and confounders. Responsive feeding-stimulation groups attained greater weight-for-age, mouthfuls eaten, hand-washing, HOME scores, responsive talking, and language. No additional benefit was derived from Sprinkles. A brief behavior change program providing on modeling and practice in feeding and stimulation was found to benefit children's nutrition and language development. Sprinkles may have been insufficient to have an effect on these malnourished children.

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ACCEPTABILITY - A NEGLECTED DIMENSION OF ACCESS TO HEALTH CARE: FINDINGS FROM A STUDY ON CHILDHOOD CONVULSIONS IN RURAL TANZANIA

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Acceptability is a poorly conceptualized dimension of access to health care. Using a study on childhood convulsions in rural Tanzania, we examined social acceptability from a user perspective. The study design is based on the premise that a match between health providers' and clients' understanding of disease is an important dimension of social acceptability. For example, childhood convulsions may not be linked with malaria and hence local treatment practices may be preferred by mothers. The present study was linked to health interventions with the objective of bridging the gap between local and biomedical understanding of convulsions. The study combined classical ethnography with the cultural epidemiology approach using the EMIC (Explanatory Model Interview Catalogue) tool. EMIC interviews were conducted in 2007/08 (n=88) and results were compared with those of an earlier study in 2004/06 (n=135). The match between local and biomedical understanding of convulsions was already high in the 2004/06 study. Specific improvements were noted in form of: (1) a 46% increase among those who reported use of mosquito nets to prevent convulsions, (2) a 2 to 13 % decrease among caregivers who associated convulsion with 'traditional causes', and (3) a 14% increase in prompt use of a health facility. Such changes can be largely attributed to interventions which explicitly aimed at increasing the match between local and biomedical understanding of malaria. The match between local and biomedical understanding of disease is fundamental for successful control and management of health problems. Health interventions should take existing local knowledge and treatment practices into account and involve communities at all stages of interventions. In return, it is clear that well ingrained traditional beliefs can be modified with communication campaigns, provided that this change resonates with the beneficiaries.

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DELIVERING INTEGRATED CASE MANAGEMENT OF CHILDREN IN UGANDA THROUGH A TWO TIER SOCIAL FRANCHISE LINKING CHWS WITH PRIVATE CLINICS

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In Uganda, most efforts to improve health of children under five are delivered via the public sector and yet recent studies show that 60-83% of the population seek initial care from private providers. Community interventions that result in early diagnosis and treatment from trained providers in the public or private sector could significantly reduce severe death and disease among children under five. PACE launched an integrated community case management program in Mubende district, Uganda in November 2010 based on village health volunteers (VHTs) referring mothers of sick children to public health facilities or private facilities forming part of an existing social franchise network. The intervention package included training of VHTs in assessing and referring sick children as well as counseling mothers in appropriate prevention and treatment practices and training and supplying private providers with subsidized treatment for malaria, pneumonia and diarrhea. VHTs serve as a link between the community and trained private providers stocked with affordable quality treatment. A management information system (MIS) was created to provide data for continuous program improvement. MIS data show increases in children under five presenting at network outlets from 296 in quarter one (Q1) to 749 in quarter two (Q2). Results on the change in the rate of treatment of children presenting with symptoms of malaria, diarrhea and pneumonia as a result of VHTs promoting increased access to affordable treatment provided through local private providers, in addition to existing public sector services will be presented and discussed.

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FACTORS ASSOCIATED WITH THE TREATMENT QUALITY FOR ILL CHILDREN SEEN BY HEALTH WORKERS TRAINED TO USE INTEGRATED MANAGEMENT OF CHILDHOOD ILLNESS (IMCI) GUIDELINES IN BENIN

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A key aim of the World Health Organization's IMCI strategy is to improve treatment of the leading causes of child deaths in developing countries. Although studies have found that training health workers (HWs) to use IMCI clinical guidelines improves treatment quality, these same studies also identified important deficiencies. To improve performance, a clearer understanding of factors influencing HW practices is critical. We analyzed >9000 outpatient consultations performed by a cohort of 32 IMCI-trained HWs in Benin. We examined associations of HW- and patient-level factors with recommended treatment (i.e., prescriptions perfectly matched IMCI guidelines) for children 2-59 months old with at least one potentially life-threatening illness (e.g., malaria/febrile illness, anemia, pneumonia, or diarrhea). Detailed assessment, diagnosis, and treatment data were abstracted from specially designed IMCI registers over a 14-month period after IMCI training in 2001. We analyzed clinical findings recorded by HWs and classified 8277 children as having at least one potentially life-threatening illness (77.2% with malaria, 34.4% with anemia, 30.4% with pneumonia, and 16.5% with diarrhea). On average, 63.7% of children received recommended treatment, although performance varied widely by individual HW (range: 14.7-87.5%). Logistic regression modeling revealed that treatment quality was significantly poorer for children: seen by older HWs (each year reduced odds of recommended treatment by 4.1%; p=0.01), >12 months old (odds ratio [OR]=0.55; p<0.0001), with more complex illnesses (OR=0.95 per additional IMCI task required [range: 18-43 tasks], p<0.0001), with a danger sign (OR=0.33, p=0.0001), and with anemia (OR=0.27, p<0.0001). Prior supervision was not significantly related to the outcome. Findings illustrate how factors outside managers' control (e.g., clinical complexity) can be important influences on performance. Quality improvement strategies, such as audit and feedback, job aids, or targeted training, which are within managers' control, should address these weak points.

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EPIDEMIOLOGICAL SURVEILLANCE OF *BURKHOLDERIA PSEUDOMALLEI, ORIENTIA TSUTSUGAMUSHI* AND *RICKETTSIA TYPHI*, USING SEROLOGY IN BANGLADESH

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Melioidosis (*Burkholderia pseudomallei* infection), Scrub typhus (*Orientia tsutsugamushi* infection*)* and Murine typhus (*Rickettsia typhi* infection) are endemic in countries in South and Southeast Asia, but this has yet to be demonstrated systematically in Bangladesh. A simple and rapid means of estimating the prevalence of exposure to these organisms is measurement of antibody levels in a representative sample of the population. A prospective, cross-sectional, hospital-based serological survey was conducted in June 2010 at 6 major hospitals in 4 of the 7 divisions of Bangladesh. Age, gender, occupation and residential address were recorded for each patient. The presence of antibodies to *B. pseudomallei* was detected using the indirect haemagglutination assay and antibodies to rickettsioses (*O. tsutsugamushi* and *R. typhi*) were detected using enzyme-

linked immunosorbent assay. Of 1,244 patients enrolled, 359 (28.9%) were positive for *B. pseudomallei*, 146 (11.7%) for *O. tsutsugamushi*, and 579 (46.5%) for *R. typhi*. Farmers had an increased risk of seropositivity to *B. pseudomallei* (RR=1.4, 95%CI 1.0-1.8,P=0.027). Seropositivity to *R. typhi* was found to be commoner in farmers (RR=4.3, 95% CI 3.4-5.4, P<0.001), service workers (RR =3.9, CI 3.1-4.9 P<0.001) and housewives (RR=1.2, 95% CI 1.2-1.4, P=0.005). Optical density of ELISA to *R. typhi* correlated strongly with age (P<0.001).There were no other associations between antibody titre and age or seropositivity and occupation, gender or residence in a rural versus urban area. There was no clear geographic clustering of seropositives. In conclusion, rates of seropositivity to *R. typhi and B. pseudomallei* in Bangladesh were considerably higher than previously appreciated. These three organisms should be considered as possible causes of undifferentiated febrile illness in Bangladesh. Further studies will be needed to establish the incidence of clinical disease and distribution of environmental risk.

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ASSESSING THE RISING CASES OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS*: HOSPITAL AND COMMUNITY-ASSOCIATED CASES

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Methicillin-resistant *Staphylococcus aureus* (MRSA) has since become a major cause of illness and death in our healthcare setting. Risk factors for HA-MRSA include hospitalization, older age, invasive devices, and residence in long-term care facility, including exposure to antimicrobial agents. HA-MRSA isolates are often resistant to several antimicrobial drug classes in addition to beta-lactams. The CA-MRSA infections usually affects young, healthy persons and associated with sharing towels or athletic equipment, participating in contact sports, living in unsanitary and crowded areas, using illegal intravenous drugs. Directions were given out for clinical microbiology laboratories to submit invasive isolates of MRSA to our unit, where we perform antimicrobial drug susceptibility tests on all isolates and characterize all isolates that were resistant to <3 non-beta-lactam antimicrobial drug classes. Most isolates were obtained from blood cultures. The full model for predicting invasive infection with CA-MRSA compared with HA-MRSA included age, seasonality, and hospital exposure, plus specimen type. The only significant predictors of CA-MRSA infection compared with HA-MRSA were age <69 years, which was associated with increased risk ([OR] 5.1, 95% [CI] 2.06-12.64), and hospital exposure (OR 0.07, 95% CI 0.01-0.51), which was associated with decreased risk. Most patients were hospitalized for their infections and the proportion of patients admitted to intensive care units did not vary by strain. Patients infected by MRSA were younger than those infected by other strains. The number of invasive MRSA infections reported and the number of invasive infections caused by CA-MRSA is on the increase. The increase of CA-MRSA poses a unique public health threat. It is now clear that CA-MRSA no longer causes only SSTIs but now causes an increased proportion of invasive infections in a rural state.

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ARE DIFFICULT-TO-REACH CHILDREN MORE LIKELY TO HARBOR TRACHOMA INFECTION?

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Mass antibiotic distributions are a major strategy for trachoma elimination. As programs in developing countries near elimination, there is concern that children who are difficult to reach may be more likely to harbor ocular strains of chlamydia that cause blinding trachoma. Here we compare infection in children who attended the initial day of monitoring versus those who presented on subsequent days. One year after administration of the third annual mass azithromycin treatment for trachoma, we performed conjunctival swabbing on a random sample of children in 12 Ethiopian communities. We defined those children who participated on the initial day as "easy-to-reach" and those who were only found on subsequent days as "difficult-to-reach." Subsequent monitoring days were necessary if all children were not present on the first day, which allows for comparison between easy-to-reach and difficult-to-reach children. Most communities required more than 1 monitoring day (10 of 12 communities). 584 children in total were assessed for the presence of chlamydial infection. On average, 15.9% (95% CI 8.8 - 26.6) of children were examined on a subsequent day. Evidence of chlamydia was found in 7.1% (95% CI 0.4 to 13.7) of children. Difficult-to-reach children were significantly less likely to have ocular chlamydial infection compared to easy-to-reach; Mantel-Haenszel common OR = 0.00 (95% CI 0.00 - 0.77). In this trachoma-endemic setting, difficult-to-reach children were less likely to harbor chlamydial infection, perhaps because those with more disease presented preferentially. This suggests that extreme efforts to achieve higher antibiotic coverage may not effectively reduce trachoma burden.

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CHARACTERIZATION OF STRAIN DIFFERENTIATION OF GENES IN *ORIENTIA TSUTSUGAMUSHI* USING MULTI-LOCUS SEQUENCE DNA TYPING (MLST)

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Orientia tsutsugamushi is the etiologic agent of scrub typhus or, more correctly, mite-borne typhus, in the Asia-Pacific region and most recently in South America. It is an obligate intracellular parasite transmitted vertically between mite generations and incidentally to humans via the bite of chiggers primarily of the genus *Leptrombidium*. Using the cell surface 56 KD gene *O. tsutsugamushi* has been well characterized as highly diverse, at least partially explaining the difficulty of developing effective vaccines. However the relationship between variation in the 56 KD type specific antigen gene and overall genome differentiation is unclear. Few studies have been reported examining the heterogeneity of more conserved housekeeping genes. This study examined the utility of multilocus sequence typing (MLST) to elucidate the diversity of *O. tsutsugamushi*. MLST was performed using PCR products amplified from purified rickettsial DNA's selected from isolates originally collected in Japan, Thailand, Burma, New Guinea, and South Korea. The strains chosen include representatives from most of the nine significant genetic subgroups within *O. tsutsugamushi* that have been identified based upon genetic differences in the 56 KD type specific antigen gene. Using the two published reference genomes, primers were developed for ten genes, i.e. *gpsA, mdh, nrdB*, *nuoF, ppdK, adk, lipA, lipB, sod*, and *groEL*. Preliminary data indicate the average pair wise distance of these genes is 2.1 per cent. This suggests that these genes may discriminate between strains and be used to construct clonal complexes within this species. Establishment of MLST protocols for *O. tsutsugamushi* could be used to characterize clinical isolates especially in regions where scrub typhus appears to be emerging.

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RISK FACTORS FOR PLAGUE MORTALITY - UGANDA, 2008-2010

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Plague is a severe, life-threatening disease. Over 95% of cases worldwide are reported from rural Africa. Although treatable, mortality rates range from 10% in developed countries to 40% in underdeveloped countries. We evaluated surveillance data and conducted a case-control study to evaluate risk factors for plague mortality in Uganda. A suspect plague case was defined as rapid onset of fever and painful lymphadenopathy or hemoptysis in a person presenting to one of the collaborating10 clinics or 2 hospitals in the plague endemic region of Uganda during January 2008 - December 2010. The case-control study, conducted during November 2008 - December 2010, included suspect plague patients and any deceased person whose death was recognized within 48 hours and suspected to be due to plague. We administered a questionnaire to study participants or their designee to assess knowledge, beliefs, attitudes, and behaviors. We compared the frequency of risk factors among patients with laboratory-confirmed plague using Chi-squared analyses. Among 199 suspect plague patients, 59 (32%) had laboratory-confirmed illness; 16 (27%) died. There were no significant differences between those who lived or died with respect to age or sex. Among 51 bubonic plague patients, the mortality rate was higher in those with cervical (4/5, 80%) vs. inguinal (9/36, 25%) manifestations (p=0.01). Twenty-six laboratory-confirmed plague patients were enrolled in the case-control study; 7 (27%) died. Patients who did not suspect plague as a cause of their illness prior to presentation or death were more likely to die (6/7, 86%) than live (7/19, 37%) (p=0.04). The median time from symptom onset to clinic presentation was 3 days and 1 day in those who died versus survived, respectively. The median travel time to the clinic did not differ between groups. Plague can be treated successfully if diagnosed early. Knowledge and suspicion of plague may result in reduced mortality in Uganda. Health care access did not appear to be a factor. These findings suggest plague education may reduce mortality in Uganda.

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ADAPTIVE IMMUNE RESPONSE TO *BRUCELLA SPP.* IN HUMANS

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Brucellosis is an ancient zoonotic disease that still represents a significant public health problem in Georgia. Infection is usually linked to exposure to infected animals and/or consumption of unpasteurized animal products. In addition, *Brucella* species are considered a substantial threat as possible biologic weapons and are included on the CDC list of possible bioterrorism threat agents. Diagnosis of brucellosis is often reliant on detecting immunological evidence of exposure to specific antigens using antibody-based blood tests. A variety of commercial kits and protocols exist for the measurement of *Brucella* specific antibodies. Despite this, our understanding of the host immune response to this disease is relatively limited and consequently there is a need for further research. In this study we examined humoral immune responses in 43 individuals diagnosed with brucellosis 3 to 12 months before enrollment, many of whom still had persistent symptoms after completion of initial therapy. Sera from 35 of 43 patients had antibodies that bound to *Brucella* lipopolysaccharide (LPS) by COMPELISA and 34 of 38 patients had demonstrable specific antibody to brucellergene OCB antigens; results from the two ELISAs were highly correlated ($p = 0.031$, $R = 0.851$). We also studied cellular immune responses in 15 patients, all of whom generated interferon (IFN)-γ in response to *ex vivo* stimulation with *Brucella* protein antigens and the majority of whom maintained measurable humoral responses to both LPS and protein antigens. From this initial study we conclude that measurement of antibody and cellular IFN-γ responses to brucellergene OCB protein epitopes may be worthy of further investigation as an alternative or adjunct to current diagnostics.

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IMMUNE RESPONSES TO PLAGUE INFECTION IN WILD *RATTUS RATTUS*, IN MADAGASCAR: A ROLE IN FOCI PERSISTENCE?

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Plague is endemic within the central highlands of Madagascar where the black rat, *Rattus rattus*, is the main reservoir. Rat immunity could play an important role in the stabilization of the foci. However, immune responses *of R. rattus* against *Yersinia pestis* are poorly investigated. Here we experimentally infected wild rats with *Y. pestis* to investigate short and long-term antibody responses. High levels of anti-F1 IgM and IgG were found in rats one and three weeks respectively after challenge, with responses differing between villages. In the long-term response experiment, a small proportion of rats had anti-F1 responses lasting more than one year. These findings may have implications for plague epidemiology. In addition, the results indicate that serological investigations in the field can detect outbreaks up to 6 months later. Comparing the ELISA and an anti-F1 antibody dipstick indicated the dipstick could be useful in the field.

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LEPTOSPIROSIS IN ACUTE FEBRILE PATIENTS IN GHANA: DIAGNOSIS BY CULTURE, SEROLOGY AND POLYMERASE CHAIN REACTION

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Leptospirosis is a zoonotic disease found in most tropical and temperate areas of the world. Humans contract Leptospira through exposure to environments contaminated by the urine of chronically infected animal sources mainly rodents, dogs, pigs and cattle. The burden of leptospirosis in Ghana is unknown or underestimated because it can mimic many diseases, e.g. malaria, dengue fever and other viral haemorrhagic diseases. In an ongoing Integrated Hospital-Based Infectious Disease Surveillance being conducted by the Ghana detachment of NAMRU - 3, in the Greater

Accra and Northern Region of Ghana, blood samples have been collected from 231 acute febrile patients meeting enrollment criteria.This study has been approved by the NMIMR and NAMRU-3 institutional review boards. 2 drops of the blood are inoculated in EMJH medium for culture and Serum is separated from the plain blood samples. So far, 180 of the serum samples have been tested for IgM antibody by ELISA and 40 for Leptospira DNA by PCR. Detection of antibody was done by Pan Bio Leptospira IgM ELISA, following manufacturer's instructions. Detection of DNA by lig-based Conventional PCR: Extraction of DNA was performed using the QIAGEN blood mini kit. Amplification of DNA: Primers used were designed from the conserved region of ligA and B. Culture results are not available now, since it needs more time. Serodiagnosis will be performed by the microscopic agglutination test (MAT). Of the 180, 14 samples (7.7%) were positive for the presence of IgM antibodies by ELISA and one was equivocal (0.55%). Conventional PCR demonstrated DNA in none of the 40 samples tested so far; also, ELISA positive samples were negative by PCR, proving PCR to be more sensitive. This suggests that almost 8% of the patients have been infected before. Leptospirosis is underreported in Ghana, where malaria is endemic, and because it is an emerging infectious disease in this part of the world, all diagnostic tools such as culture, MAT, ELISA, Conventional and Real time PCR (RT-PCR), should be explored to know the burden of this disease. In addition, improve treatment of patients in Ghana.

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ANTIBIOTIC RESISTANCE OF INVASIVE NON-TYPHOIDAL *SALMONELLA* (NTS) ISOLATES IN CHILDREN FROM WESTERN KENYA

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Community-acquired non-typhoidal *Salmonella* (NTS) bacteremia is widespread in *Plasmodium falciparum* holoendemic transmission regions of Africa. In these regions, NTS bacteremia complicates malaria and other childhood illnesses, and increases childhood mortalities. Our recent studies have shown that NTS is the most common cause of malaria-related bacteremia and enhances malaria severity and mortality in children from western Kenya. Clinical treatment of children with NTS infections is worsened by the rampant and increasing antimicrobial resistance. As such, the patterns of antibiotic resistance by bloodstream NTS isolates were investigated in children (n=67) from western Kenya with *P. falciparum* malaria (n=30), without malaria (n=20) and following acute febrile visits to hospital (n=17; malaria[+], n=1 and malaria[-], n=16). *Salmonella enterica* serotypes Typhimurium and Enteritidis were the only serotypes isolated using standard microbiological procedures, while *in vitro* antibiotic resistance defined by intermediate or full resistance was determined using the disc diffusion method. Results reveal that *in vitro* resistance to ampicillin/salbactum [60/67 (89.6%)]; amoxicillin/clavulanic acid [50/66 (75.8%)]; trimethoprim/sulfamethoxazole [58/67 (86.6%)]; chloramphenicol [52/67 (77.6%)]; ciprofloxacin [22/43 (51.2%)]; cefotaxime/clavulanic acid [9/29 (31.0%)]; nalidixic acid [7/30 (23.3%)] and gentamicin [14/64 (21.9%)] was common. Multi-drug resistance, based on resistance to three or more antibiotic classes, was also high [56/67 (83.6%)]. Additional analyses demonstrated higher ciprofloxacin resistance in children without malaria [13/15 (86.7%)] relative to those with malaria [7/18 (38.9%); P=0.011] and acute visits [2/10 (20.0%); P=0.002]. These results demonstrate that antimicrobial resistance to common antibiotic agents is high in this area, justifying an urgent need for clinical and public health surveillance.

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CHARACTERIZATION OF EXTREMELY DRUG RESISTANT ISOLATES OF *MYCOBACTERIUM TUBERCULOSIS* DETECTED IN COLOMBIA DURING 2006 TO 2010

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Extremely drug resistant tuberculosis (XDR-TB) represents an important threat to TB control worldwide. Resistance to isoniazid and rifampin, the two main drugs used for TB treatment, together with resistance to a fluoroquinolone and one of three injectable second-line anti-TB drugs, makes the XDR cases difficult to treat and cure. According to the World Health Organization, Colombia reported at least one XDR case since 2008, and nine XDR-TB cases have been detected in the past five years in Valle del Cauca. The aim of this study was to perform a molecular characterization of this set of XDR isolates, including a sociodemographic description of cases. XDR profile was identified using Proportion Method on 7H10 agar medium. Sociodemographic data and clinical outcome were obtained through local health authorities. The isolates were genotyped using spoligotyping and MIRU-VNTR 24 loci methodologies and mutations associated with resistance to first and second-line anti-TB drugs were detected using Genotype® MTBDR*plus* and *sl* assays (Hain lifescience). The majority of patients were male (56%) and patient's age ranged from 16 to 44 years with a median age of 30 years. Five out of the nine patients had a mortal outcome (56%). Spoligotyping identified three families: Beijing 190 (56%), H1 62 (11%), U 881 (11%) and two different orphan types (22%). All isolates were further classified into 7 genotypes using MIRU-VNTR. Moreover, three of the mortal cases were classified as Beijing 190, clustered as the same MIRU-VNTR genotype and also had the same mutations for the evaluated genes, which could suggest clonality. The S315T1 mutation in the *katG* gene for resistance to isoniazid and the S531L mutation in the *rpoB* gene for rifampin resistance were the most frequent (89% and 67%, respectively). D94G, A1401G and M306V mutations were found in the *gyrA*, *rrs* and *embB* genes (67%, 100% and 56%) associated to fluoroquinolones, aminoglicosides and ethambutol resistance, respectively. The high frequency of *katG* S315T1 and *rpoB* S531L mutations are consistent with previously reports from other South American countries. Genetic screening of these mutations may provide rapid detection of XDR cases and improve their treatment. The mortal Beijing 190 cluster identified in this study calls for further detailed studies including virulence factors, which may lead to novel drug targets.

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SEROTYPING INVASIVE PNEUMOCOCCAL MENINGITIS IN THE REGION OF BOBO-DIOULASSO

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Meningitis remains a major public health problem. The western Burkina Faso is in the African meningitis belt, with recurrent epidemics of bacterial meningitis. These outbreaks are usually caused by Neisseria meningitidis, a strainable alone to cause epidemics of meningitis. However, in recent years there has been an upsurge in cases of meningitis due to Streptococcus pneumoniae that occur throughout the year with a fatality rate of pneumococcal meningitis (~ 50%) 5-10 times higher than for Meningococcal meningitis in [7], but unfortunately very few studies show serotype profile of this germ in Africa. Our study aims to profile the seeds of pneumococcal serotypes circulating in the area of western Burkina Faso. Its main objective to participate in the microbiological monitoring of pneumococcus by monitoring the emergence of new types of germs, and the resurgence of invasive strains in the region of the high-basins. We also study the circulation and the biodiversity of strains of S. pneumoniae infections of pneumococcal meningitis in the western region of Burkina Faso. Patients with suspected meningitis were recruited between 2009 and 2010. Samples of cerebrospinal fluid were collected and analyzed by

standard microbiological techniques. Bacterial isolates were analysed by PCR. An increase in the incidence of pneumococcal meningitis has been observed from 2009 to 2010. Of the 154 samples of S. pneumoniae analyzed, the serotype 1 represented for more than 50% of germs from CSF analyzed with a high virulent lineage and a high propensity to cause meningitis; our results suggest that this strain may have the potential to cause an epidemic. Conclusion: These preliminary results show that the growing of this strain could be a potential epidemic. As perspective we need to investigate the virulence of this serotype 1 and others serotypes on the ability of invasive pneumococcal disease.

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MIXED INFECTION OF *CHLAMYDIA TRACHOMATIS* GENOTYPE L2 AND *CHLAMYDIOPHILA ABORTUS* IN PIGS

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In Ukraine, chlamydiosis is diagnosed in 73% from studied pig samples. In the first-time impregnated pigs the abortion rate is 45%; abortions in master sows are rare, they get usually 2-3 still piglets, lethality of others is 60%. Three aborted foetuses from the pig farm in Cherkasy region, Ukraine, were studied using microscopy and IFT. 5 day chicken embryos (CE) were charged with specimens of aborted foetuses. Experimental infection was performed using white mice, guinea pigs, and gnotobiotic piglets. CE, guinea pigs and gnotobiotic piglets were studied in genus-specific RT-PCR and microarrays (Alere) for detection of chlamydial species and genotyping of *Chlamydia* trachomatis. The pig farm had previous history of chlamydiosis for about 3 years. The dissection of aborted foetuses revealed hyperemia of brain vessels and liver dystrophy, petehia and spread haemorrhages in epicardium and kidneys. Microscopy and IFTs were positive. Chlamydiae (strain A-2536) were isolated on CE. The 6th CE passage induced death in 4.2 d.p.i. with the infectious titer 10-4,16 ELD50/0.4ml. The 5th and 4th CE passages caused 100% lethality in white mice, and 30-40% lethality and 100% abortions (in 5-6 d.p.i) in guinea pigs respectively. The 5th passage of isolated chlamydiae on chicken embryos did not cause visible clinical signs in gnotobiotic piglets in 35 d.p.i. with exception of 1 piglet with acute disease which dead on 21 d.p.i. Multiple pathological changes typical for chlamydiosis were found in dissection. Chicken egg yolk, organs of guinea pigs and gnotobiotic piglets were positive in genus-specific RT-PCR. Using the same DNAs, the microarray assay revealed mixed infection of *C. trachomatis* and *Chlamydophila* spp. in chicken egg yolk; *C. trachomatis, C. abortus*, and *Chlamydia* and *Chlamydophila* in 1 guinea pig; *C. trachomatis* and *Chlamydia* but no *Chlamydophila* in all gnotobiots. Microarray based genotyping of DNA from gnotobiots revealed genotype L2 of C. trachomatis.

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DEVELOPMENT OF ELISA FOR THE DETECTION OF LEPTOSPIROSIS SPECIFIC ANTIBODIES USING THE OUTER MEMBRANE LIPOPROTEIN LIPL32 AND LIPL41

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Leptospirosis is caused by spirochaetes of the genus *Leptospira*. It is considered to be the most widespread zoonotic disease in the world. Symptoms of leptospirosis are easily confused with a variety of other febrile illnesses (e.g., dengue and malaria) that require different treatment regimens. Currently, the Microscopic Agglutination Test (MAT) is the standard method for the diagnosis of leptospirosis. It is not only technically complex but also time-consuming. With the publication of the whole genome sequences of several pathogenic species of *Leptospira*, hundreds of genes encoding surface-exposed lipoproteins and outer membrane proteins were identified as candidates for the development of rapid diagnostics of leptospirosis. Among them, LipL32 and LipL41 are considered excellent candidates as they are present only in pathogenic strains and have been shown as surface exposed. Here, we prepared recombinant LipL32 and LipL41 proteins and showed that both were recognized by leptospirosis patient sera in western blot. Fifteen MAT confirmed positive sera were used to evaluate these two antigens in ELISA. Preliminary results showed 9 were IgG and 9 were IgM positive against LipL32. Eleven samples were IgG positive and 10 samples were IgM positive against LipL41, respectively. Some samples had specific IgM antibody against LipL32 only and some had specific IgM against LipL41 only but not both. These data suggested that both LipL32 and LipL41 antigens should be needed to improve the assay sensitivity and to develop rapid sero-diagnostic assays.

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HOW RESISTANT IS *STAPHYLOCCOCUS AUREUS* IN PEDIATRICS AT PONCE SAN LUCAS HOSPITAL?

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Community Acquired Staphylococcus aureus (CA-MRSA) infections, are usually, acquired by community persons without hospitalizations or surgical interventions during the year preceding the infection. Infections with CA-MRSA were first reported in the United States pediatric population during the 90's, mainly as a cause of skin and soft tissue infections. Frequency of CA-MRSA infections in Pediatric patients admitted to Hospital Episcopal San Lucas is unknown. Data shows that these infections doubled from 33% to 65% during 2004 to 2006. The objectives of our study were to determine resistance patterns of CA-MRSA skin and soft tissue infections, and associated morbidity in pediatric population admitted to hospital. This is a descriptive, transversal retrospective, IRB approved study was done. Cultures from all pediatric patients admitted to hospital (59) from January to December of 2008 were reviewed. Exclusions were made for infections other than those of skin and soft tissue, nosocomial or immune-compromised children (28). Localization of infection, antibiotics used, and changes in therapy were obtained (29). Our findings includes; children from 6 months to 3 years were more frequently admitted (13 cases/44%). In our sample almost 60% of admitted children with skin and soft tissue infections had CA-MRSA as per sensitivity pattern. A large portion of community physicians, still use penicillin derivatives, or first generation cephalosporins before or after lesion drainage even though CA-MRSA is highly resistant to these antibiotics. We conclude in our study that CA-MRSA is a frequent cause of skin and soft tissue infections in the pediatric population admitted to our hospital. Children ≤ 3years are most frequently affected. The high rate use of penicillin derivatives or first generations cephalosporins for treatment of skin and soft tissue infections by CA-MRSA, needs to be addressed, so community physicians become aware of the recommendations issued for proper management of these infections.

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EXPERIMENTAL LEPTOSPIROSIS IN HAMSTERS INDUCE HYPOMAGNESEMIA AND HYPERKALEMIA IN ACUTE-PHASE DISEASE

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Patients with severe leptospirosis develop hypomagnesemia during acute phase disease as well as sodium and potassium wasting defects. Non-oliguric renal failure rapidly evolves to an oliguric hyperkalemic form that is associated with a poor outcome. The renal waste of Mg2+, Na+, and K+ may be related to the production of nitric oxide (NO), which is

a known inhibitor of the Na,2Cl,K co-transporter of the thick ascending limb. Hamsters were experimentally infected with the virulent Leptospira interrogans serovar Copenhageni strain Cop (~ 6 fold the lethal dose 50%). Groups of five hamsters were euthanized at different intervals (4, 8, 16 and 28 days post-infection) and evaluated for kidney lesions and serum levels of NO, K+, Mg2+ and Na+. Hamsters were separated into four groups according to the treatment regimen started on the tenth day: ampicillin (AMP), methylene blue (MB), ampicillin and methylene blue (BOTH) and no treatment (NONE). MB is a known inhibitor of nitric oxide synthase. All groups exhibited increasing serum levels of K+ from day 4 to day 16. In addition, a significant decrease in the serum levels of Mg2+ was observed in all groups on day 8. Serum levels of Na+ remained unaffected by the treatment regimens, except for a decrease in the MB group on day 16. In conclusion, hamsters developed hypomagnesemia during the acute phase of experimental leptospirosis, which was not prevented by antimicrobial treatment or inhibition of NO production. Conversely, hypokalemia was not observed, as all groups showed increasing levels of serum K+. Furthermore, treatment with MB had no effect on Mg2+ and K+ serum levels during acute phase leptospirosis in hamsters.

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BACTEREMIA AND ANTIBIOTIC RESISTANCE IN ACUTE FEBRILE PATIENTS IN ACCRA, GHANA - A PILOT STUDY

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Malaria is a leading cause of morbidity in Ghana, accounting for 40-60 percent of cases in outpatient clinics. Unfortunately, malaria diagnosis is often inferred from subjective presenting symptoms rather than objective laboratory results. It is important therefore to establish the burden of non-malarial pathogens in acute febrile illness. The pilot study being described herein was undertaken to describe the burden of disease presenting as febrile illness in two Accra hospitals. One hundred and sixty four people were enrolled in this surveillance study. Patients presenting with fever lasting two days or more and a temperature of >38°C were recruited. Individuals with an obvious focal clinical diagnosis like diarrhea, respiratory or urinary tract infection, cellulites and or rheumatic fever and children under the age of 4 years were excluded. Enrolled cases were those that had met the case definition and given informed written consent. This study was approved by the Noguchi Memorial Institute for Medical Research and Naval Medical Research Unit-3 institutional review boards. Data on sex, age and recent exposure to rodents, pets or domestic animals was recorded and 7-10 ml of venous blood drawn for serology, culture and malaria thick film tests. All isolates were identified, while the malaria film results were obtained from the hospital laboratories. A total seven (4.27%) bacteria were isolated and identified; two *Salmonella typhi,* one group A *Streptococcus,* one Salmonella Group B and three *Staphylococcus aureus*. One of the bactaeremia positive blood samples was malaria smear positive, while five were negative and one was not tested. The clinical diagnosis for five of these was malaria. Four isolates out of the eight were found to be resistant to Ampicillin while one *S. aureus* showed resistance to Ampicillin, Penicillin and Oxacillin. The *Salmonella* group B isolate was resistant to Chloramphenicol, Ampicillin and Sulfamethoxazole/ Trimethoprim. This information suggests that bacterial infections are responsible for at least one out of every twenty five presumed malaria cases. Additionally, viral etiologies ought to be given consideration when patients with febrile disease present at our hospitals. In order to get a clearer picture of agents of febrile disease in Accra, a larger study which includes additional hospitals, evaluation for viral pathogens and children under the age of four, is in the process.

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PREVALENCE AND RISK FACTORS OF LEPTOSPIROSIS AMONG RICE FARMERS OF ENDEMIC AREA IN A TROPICAL REGION OF PERU

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Leptospirosis is a zoonotic infection of major impact in tropical regions. We conducted a seroepidemiological cross-sectional study in the Valle del Alto Mayo, an endemic area of leptospirosis in the Peruvian Amazon, in order to identify the prevalence, local serovars, and associated risk factors of leptospirosis. 261 rice farmers were randomly selected to participate. Overall, leptospirosis infection was found to be 64.75% (95%CI: 58.76-70.74). The prevalence of specific serovars identified by microscopic agglutination (MAT) included: *Leptospira icterohaemorrhagiae* (34.5%), *L. autumnalis* (19.5%), *L. panama* (13.0%), *L. australis* (12.6%), *L. grippotyphosa* (8.4%), *L. bataviae* (5.4%), *L. djasiman* (4.9%), *L. pyrogenes* (3.8%), *L. cynopteri* (2.3%), *L. pomona* (1.9%), *L. georgia* (1.5%), *L. canicola* (1.1%), for *L. borincana*, L. *bratislava*, *L. ballum*, *L. wolfii* and *L. javanica* (0.76%), and *L. varillal* and *L. harjo* (0.38%). Among 169 positives cases, 84 (49.7%) were positive for one serovar, 58 (34.3%) for 2, and 27 (16.1%) to 3 or more. In addition, 35 of 169 (20.7%) positives cases were IgM positive, meaning they were in the acute phase of infection. Risk factors of infection included male sex (OR = 3.16, p=0.001), age between 30 to 49 years (OR = 1.86, p=0.001), a low level of education (OR = 1.67, p=0.03), working barefoot (OR = 1.84, p=0.001), and handling rats in the field (OR = 2.24, p=0.003). In conclusion, there is a high prevalence of leptospirosis among rice farmers of the Peruvian Amazon, of whom many were positive for more than one serovar. It is necessary to implement prevention and control of leptospirosis in this region.

1100

METALLOBETALACTAMASES PRODUCING *ENTEROBACTER* SPP. STRAINS FROM THE CENTRAL HOSPITAL OF CUMANÁ, SUCRE STATE, VENEZUELA

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Carbapenems represent the therapeutic of choice in hospital-acquired infections caused by multiresistant Enterobacteria. However, metallobetalactamases (MBL)-producing bacterial strains, which are resistant to carbapenems, are increasing their frequency worldwide and constitute a threat to the health of patients, especially in developing countries. From the period of August 2010 and March 2011, three Enterobacter spp. resistant to imipenem and meropenem were isolated from the central Hospital of Cumana (HUAPA), Sucre state, whose susceptibility was assessed by the Kirby-Bauer disk-diffusion assay. The double-disc synergy test with Imipenem/Meroperem and EDTA-Sodium thioglycolate showed the presence of MBL in all of the strains of *Enterobacter*. These strains were also resistant to most betalactams, ciprofloxacin and trimethoprim-sulfamethoxazole, although two of the strains were sensitive to aminoglycosides and all of them were sensitive to tigecycline. Amplification by PCR of a 382 pb fragment, using primers specific for VIM-type MBL gene on the DNA isolated from those three strains, showed the presence of this gene. The amplification using both VIM-1 and VIM-2 types showed the expected fragment of 801 bp only for the VIM-2 type in all the strains. As far as we know, this represents the first report of a MBL-producing *Enterobacter* strain and the first report of VIM genes in this genus in Venezuela and only a few cases of infection

due to MBL-producing *Enterobacter* have been reported in the literature worldwide. This represents a major matter of concern for hospital authorities since Enterobacter spp. are significant causes of nosocomial infections and are intrinsically resistant to aminopenicillins, cefazolin, and cefoxitin due to the production of constitutive chromosomal AmpC betalactamases.

1101

MULTIPLE ANTIBIOTIC RESISTANCE IN CLINICAL ISOLATES FROM GHANA

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The ever increasing resistance to antibiotics is a serious worldwide problem which has implications for morbidity, mortality and health-care both in hospitals and in the community especially in a developing country such as Ghana. This study, therefore, looks at an *in vitro* antibiotic sensitivity pattern of 7 standard bacteria and 14 wild-type bacteria isolated using Kirby-Bauer disc diffusion method and the guidelines set by the National Committee for Clinical Laboratory Standard. Briefly, two to six hour cultures of the microbes in peptone water that had achieved the 0.5 McFarland standard turbidity were flooded over Mueller-Hinton agar and antibiotic disc aseptically placed on the surface of the agar, allowed to dry, before being incubated at 37 ºC for 16-18 hours. The antibiotics tested included; Amikacin (30 µg/disc), Ampicillin (10 µg/disc), Penicillin (10 iu/disc), Cloxacillin (5 µg/disc), Erythromycin (15 µg/disc), Tetracycline (30 µg/disc), Gentamicin (10 µg/disc), Cotrimoxazole (25 µg/disc), Chloramphenicol (30 µg/disc), and some of the newer generation antibiotics including Cefixime (30 µg/disc), Cefuroxime (30 µg/disc), and Cefotaxime (30 µg/disc). The study revealed that, 29% of the isolates were resistant to all the 12 antibiotics used, 14% were resistant to 10 antibiotics, 21% were resistant 9, 25% were resistant to 8, 8% were resistant 6 while 4% were resistant to 5 antibiotics. Interestingly, all the microbes were resistant to tetracycline, cloxacillin, ampicillin and penicillin while 91.7% were resistant to erythromycin, all being first-line antibiotics in Ghana. Thus, a very serious multiple resistant antibiotic pattern of bacteria exists in Ghana.

1102

AN EXPLORATION OF THE KNOWLEDGE, ATTITUDES AND PERCEPTIONS OF THE LOCAL, ADULT, NON-MEDICALLY TRAINED GRENADIAN POPULATION ABOUT CERTAIN ZOONOTIC DISEASES

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Zoonotic diseases represent a leading cause of illness and death from infectious diseases in humans. The objective of this research study was to explore the knowledge, attitudes and perceptions of the local, adult, non-medically trained Grenadian population about certain zoonotic diseases. The study consisted of a quasi-experimental design consisting of 450 participants, selected using a convenience sampling in the Grand Anse and the Carenage areas of St. George's, Grenada. A questionnaire was employed to collect data on the knowledge, attitudes and perceptions towards five zoonotic diseases (ringworm, leptospirosis, creeping eruptions, rabies and salmonellosis). The overall level of distribution of knowledge of zoonotic diseases was 38.6%. Knowledge of Ringworm (81.0%) was predominant among participants while leptospirosis and creeping eruption demonstrated the greatest deficiency in participants' knowledge. Knowledge of zoonotic diseases was found to have an effect on the attitudes and perceptions of persons towards the diseases. Education (p=0.0000) and income (p=0.0000) were found to be determinants of zoonotic disease knowledge while age (p=0.56) and gender (p=0.97) had negligible influence on the measure of knowledge, attitudes and perceptions.

1103

THE GEOGRAPHY AND ECOLOGY OF ANEMIA IN THE DEMOCRATIC REPUBLIC OF CONGO

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Anemia is a severe public health problem in the Democratic Republic of Congo (DRC). A better understanding of the spatial distribution of anemia as well as its causes can help the government to focus its prevention strategies. Using hemoglobin levels reported in the 2007 Demographic and Health Survey (DHS) for the DRC, prevalence estimates were generated and ecological drivers of malaria were explored using spatial statistical analyses and multilevel modelling. Of the 4638 female respondents aged 15-59 years, 29% were anemic (hemoglobin <11 g/dL); of the 526 pregnant respondents, 53% were anaemic. Regional variation in these rates was mapped using the inverse-distance weighting spatial interpolation technique. Pregnant women were 33% more likely to be anemic (p<.0001). Certain ethnic affiliations were associated with increased risk for anemia in pregnant women (p<.05). Older women (p<.05) were at increased risk, while pregnant women owning a refrigerator were 32% less likely to be anemic than other pregnant women (p<.05). Living in certain agricultural zones was protective while others increased risk for anemia. Neither malaria PCR positivity nor HIV seropositivity increased the risk of anemia. This research demonstrates the feasibility of using population-based demographic data and geographic methods to study nutritional deficiencies in a tropical setting. This study provides the most accurate population-based estimates to date of where anaemia occurs in the DRC and what factors contribute to the estimated spatial patterns.

1104

NOMADS ACCESS TO THE CURRENT HEALTHCARE SYSTEM IS IMPAIRED BY THEIR PERCEPTION OF ITS COST, QUALITY, ACCESSIBILITY AND BY GENDER SEGREGATION IN TIMBUKTU, MALI

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Access to community-based healthcare services is one of the key factors in successful public health policy. In Mali, community-based interventions do not reach nomadic communities because of their lifestyle. In order to determine a better healthcare strategy for these nomadic populations, we conducted a cross-sectional survey in the administrative region of Timbuktu in Mali consisting of interviews of key members of the communities and distribution of questionnaires to community members, health care providers, traditional healers, community leaders and the mothers of children of 5 years or less. Informed consent was obtained from all participants. A mixed quantitative and qualitative data analysis approach was used. A total of 520 individuals from two nomadic communities, Gossi and Ber, were included in the questionnaire survey. Twenty (4%) underwent an additional interview. Based on the questionnaire survey, inhabitants of the two nomadic communities were livestock breeders (27%), housekeepers (26.4%), local traders (11%), farmers (6 %) and artisans (5.5%). The median age of the study subjects

was 38 years (18-86 years). The participants from Gossi and Ber lived a mean distance of 22.4 km and 8 km from the closest health center, respectively. The major complaints with respect to healthcare access were cost (35.7%), distance to the health center (46.2%), the quality of the services provided (39.2.%) and the lack of finances or means of displacement (79.4%). About 67% of the participants visit traditional healers first when they are sick. More than 25% of the participants from the community stated that they will never accept to be examined by a health care provider of the opposite gender. In summary, it appears from the interviews that the nomadic population has health needs not covered by the current health delivery system. Tackling the method and organization of health care delivery by adapting them to the local lifestyle, culture and values could lead to significant improvements in this regard.

1105

CURRICULUM DEVELOPMENT AND OVERSEAS OPPORTUNITIES IN TROPICAL INFECTIOUS DISEASES FOR THE 21ST CENTURY

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A needs-based analysis of our curriculum for graduate and medical students demonstrated that the Department's curriculum was centered on approaches to infectious disease more appropriate for developed countries. However, our graduate students are keenly interested and motivated to learn more about infectious diseases in tropical areas. JABSOM's Problem Based Learning Curriculum for medical students emphasizes clinical reasoning with limited exposure to global health issues. Opportunities for international research experiences for medical and graduate students were limited. With this in mind, we set out to reorganize our infectious disease curriculum. We are in the final phases of developing a one-year certificate course that covers didactic, laboratory and clinical aspects of infectious diseases. Physicians completing the Department's certificate course and who have completed a 2-month clinical overseas rotation will be eligible to qualify for the CTropMed Diploma examination offered by the American Society of Tropical Medicine and Hygiene. The core courses for the master's and doctoral degrees have been reorganized and can be completed during the first year after admission to the program. We have partnered with the other departments to provide learning opportunities in epidemiology, biostatistics, nutrition, maternal-child health, water supply/waste water management, and sanitation. Our pharmacology faculty is participating in all aspects of infectious diseases including treatment, control, and the development of new drugs. The immunology faculty is emphasizing the interplay of the host with the infectious agent in the development of immunity and/or disease along with the theory and practicalities of vaccine development. Finally, overseas opportunities have been set up to provide field experiences for graduate students, medical students and practicing physicians in the Asia-Pacific. We hope the exchange of students and faculty will foster a greater cultural understanding of infectious diseases in the context of real world experiences.

1106

ANTIBIOTIC THERAPY AND HYGIENE MEASURES TO INTERRUPT CHOLERA TRANSMISSION IN A PRISON IN ST. MARC, HAITI

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Partners In Health (PIH) supports the Ministry of Health (MOH) in Haiti to provide comprehensive health care to the rural and urban poor. In October 2010 cases of acute fatal watery diarrhea amongst adults in St Marc, Haiti signaled the beginning of a cholera epidemic that is currently ongoing. Living conditions in the St Marc prison are severely overcrowded and sanitation conditions are very poor. PIH is engaged in long-term medical mobile clinic activities in the prison in support of MOH. This abstract describes the initial cholera epidemic in the prison. On November 14th 2010 St Marc prison reported the first inmate with cholera, he died the next day. On that day, the prison housed 315 inmates and during the peak of the epidemic held 411 prisoners in 14 cells, each approximately 4 meter [2]. A mobile team from PIH comprised of three physicians, one public health coordinator and two community health educators collaborated with prison and MOH authorities to intervene in both treatment and prevention roles. Treatment: 16 cases of cholera were diagnosed on the teams arrival; a cholera treatment unit was established inside the prison with two cells transformed into cholera wards. An inmate at the prison was a trained nurse's aide and she was engaged to provide overnight care of the patients. Prevention: Bottled water was provided to inmates and later water purification tablets were used for the prison well water; cleaners were sent daily to clean and spray the prison with 0.2% chlorine spray; doxycycline 300mg was prescribed as a single dose by mouth to all inmates and prison guards once per week for a period of one month; training for prison guards and health promotion activities were carried out in the prison. In the four weeks following the intervention, there were no subsequent deaths in the prison of cholera. A total of 26 cases were registered. Treatment activities continued for one month until no new cases occurred. Early in the course of a cholera epidemic, close attention should be paid to hygiene and sanitation in prison settings to avoid unnecessary deaths. Antibiotic therapy and intense hygiene measures interrupted the initial cholera outbreak in St Marc prison.

1107

NOT AS SIMPLE AS IT SOUNDS: EVALUATION OF A BEHAVIOR CHANGE COMMUNICATION (BCC) INTERVENTION TO INCREASE PROMPT AND EFFECTIVE MALARIA TREATMENT IN CHILDREN UNDER FIVE IN KENYA

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In 2009, Population Services International in collaboration with the Ministry of Public Health and Sanitation launched a malaria behavior change communication intervention in Bondo district, Nyanza Province. The initiative aimed to improve: symptom recognition and prompt access to government clinics for febrile children; effective treatment with the recommended first-line drug artemether-lumefantrine (AL) in public health facilities; and adherence to the AL regimen by the child's caregivers. The intervention used various communication channels: road shows, radio spots, print media and community outreach to deliver 10 key messages. It was implemented between October 2009 and September 2010 and was evaluated through pre-post-intervention cross sectional household surveys. The surveys were undertaken in June/July2009 and July/August 2010. Households were selected using multi-stage cluster sampling and included in the survey if there was a child under 5. The primary outcome was the proportion of children under 5 with fever in the last 14 days accessing AL within 48 hours of fever onset. Logistic regression was used to test the association between the intervention and primary outcome. In the pre-intervention survey 600 households were surveyed giving 628 mothers with 958 children under 5 of whom 506 had been febrile in the past 14 days. In 2010, 700 households were surveyed containing 717 mothers with 1023 children under 5 of whom 515 had been febrile in the previous 14 days. The proportion of caregivers who sought any treatment for their febrile child within 48 hours increased between surveys [62.8% (59.1-66.4) vs 79.4% (74.8-83.3)]. However, there were no significant increases in the proportion of children accessing AL within 48 hours of fever onset [18.4% (15.0-22.3) vs 23.5% (19.5-28.0)] and there was a significant decrease in the use of government clinics. Logistic regression

on the 2010 data showed no association between exposure to the intervention messages and the primary outcome, however, knowledge of AL as the recommended treatment for uncomplicated malaria in children was significantly associated with prompt access to AL (OR: 4.3; 95%CI: 1.4, 12.7). The implications of these findings for the evaluation of BCC interventions, the relationship between knowledge and behavior and the complexity involved in attaining the roll back malaria target of 80% of malaria cases receiving prompt treatment with AL are discussed.

1108

COLLABORATION ON THE CONSTRUCTION OF A CLINICAL SITE FOR GLOBAL HEALTH EXPERIENCES

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Global Health and international medicine programs often take place in areas of the world that have limited access to health care services and scarce local resources which may require building rather expensive infrastructure to support the local program. Establishing partnerships is essential for the success of the initiative and to ensure sustainability. As part of the Global Health Honduras program of the Department of Family Medicine in the School of Medicine, and the developing Physician Assistant Program in the School of Health and Rehabilitation Sciences, both at Indiana University in Indianapolis, a clinical site is being built in collaboration with local Honduran, as well as American partners, both public and private. Construction is taking place on a donated property and work is done utilizing local resources by local laborers, which has brought some income to many of the families of the surrounding villages. International partners include the academic medical center, several charitable 501(c)3 organizations, and many individuals who have contributed time and resources towards its success. The soon to be completed medical center will serve multiple purposes: Besides being a clinical site for much needed local patient care and an international medical and health care education training center, it will also serve as a "communities center" to enhance the existing collaboration with local public health; and also as a hub for a communication and epidemiological surveillance center for surrounding isolated mountain villages which are medically under-served. Clinical care, health education and research to improve the quality of life for these residents is ongoing. This model of collaborative construction and future operation to serve the many needs of both local care and international medical and health care education is innovative as to address multiple needs, including enhancing interest in medical and other health care student's involvement in global health and international service learning, as well as involving multiple cooperating supporting agencies.

1109

MYSTERY CLIENTS AND DRUG COCKTAILS: FINDING OUT WHAT PATIENTS ARE REALLY BEING SOLD

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In Cambodia the first source of treatment for fever and other illnesses is often the nearest private provider - a private pharmacy or clinic, or most often, a informal drug seller. Previous studies have documented that the drugs are most often dispensed as little plastic bags containing a colourful "cocktail" of several different tablets and capsules. These appear to often contain antibiotics and antimalarials as well as antipyretics and vitamins, however their actual content has remained unknown. In order to identify accurately the contents of drug cocktails and to measure the frequency with which these are sold, we carried out a "mystery client" study. Actors presented to private providers with malaria-like symptoms, purchased drugs that were offered and documented the details of interaction, including whether or not they were offered a blood test or antimalarials. Over 200 interactions took place in 12 districts across Cambodia and the contents of the purchased cocktails are being analysed by mass spectrometry. We present the results of the analysis and discuss the implications for antimalarial and antibiotic resistance and patient safety as well as recommendations for policy and practice.

1110

POLIO ERADICATION IN PAKISTAN - THE LAST FRONTIER

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Continued poliovirus transmission in Pakistan poses a significant challenge to the Global Polio Eradication Initiative (GPEI). Pakistan has reported more polio cases than all other endemic countries combined for two years in a row. This burden is concentrated in a single geographical zone in the country's north-west, the Federally Administered Tribal Areas (FATA), reporting 74 (51%) cases in 2010. Military conflict in FATA has hampered immunization activities since 2008. Genetic homogeneity between viral isolates from FATA compared to 2 non-adjacent zones of persistent transmission, and other districts with recent outbreaks reaffirm this region's major polio reservoir status and highlight the presence of susceptible populations elsewhere in the country. We are conducting a comprehensive review of the Polio Eradication Initiative (PEI) in Pakistan, including a quantitative model, to explore reasons for failure at the district level. Pakistan reported 144 cases of polio in 2010 and 40 cases by May 23, 2011. An analysis of polio cases reported during 2009-2011 showed that 71% of cases had received no routine OPV doses and 32% had received no supplementary OPV (compared to 49% and 20% respectively in 2000-2002). Field observations of polio vaccination campaigns in Karachi showed many poorly motivated and under-paid vaccinators (paid only $1.7 per day), adolescents and children employed as vaccinators, variable quality of independent monitoring and lack of prior campaign publicity. Failure to vaccinate is the dominant explanation for continued transmission of polio in Pakistan. Lack of progress in polio eradication in Pakistan will lead to failure of the GPEI Strategic Plan 2010-2012. Heroic efforts to establish negotiated peace in FATA, massive increase in resources at the ground level, and involvement of local non-governmental partners to develop specific solutions for poorly performing areas are needed urgently if the goals of global polio eradication are to be met early in this decade.

1111

HOUSEHOLD EXPENDITURES DUE TO MALARIA CASE MANAGEMENT - UGANDA, 2009

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Malaria is the most frequently reported disease at both public and private health facilities in Uganda. Nearly 10 million cases of probable or confirmed malaria, the majority of which are in children under 5, were reported in 2009. Although access to effective treatment, including ACTs, has improved, such therapies are expensive and impoverished individuals continue to be disproportionately affected by illness. The RBM Partnership indicates that malaria "imposes a harsh economic burden on families who are least able to pay". The 2009 Uganda Malaria Indicator Survey (MIS) is a nationally representative household survey which provided coverage estimates of prevention and control activities. To assess the economic impact of malaria, we analyzed data on household malaria expenditures. We calculated the costs incurred for caring for a febrile child during the previous two weeks and performed both univariate and multivariate analyses. The MIS utilized a two-stage sample design; 4,421 households were randomly selected (response rate 97.5%) from 170 clusters. Of the

1667children under 5 with a reported fever in the prior 2 weeks, 1366 (81.9%) received medical care. Of these, 210 (15.4%) paid money for transportation, 394 (28.9%) for consultation, 758 (55.5%) for medicine, and 41 (3.0%) for hospitalization, with a mean total expenditure of $5.42 USD. The mean expenditure for ACTs ($2.54 USD) was lower than for other anti-malarial therapy ($3.21). A total of 230 (16.8%) had to borrow funds, 302 (22.1%) had to sell items, and 596 (43.6%) of caregivers took time off from their normal duties to care for their ill child (=4.87 days). Costs incurred at private and public health facilities were similar. Households in urban areas (p <.005) and belonging to higher wealth quintiles (p <.005) spent more money than their counterparts. Despite efforts to increase access to provide free and effective therapy in Uganda, the majority of caregivers incurred costs to care for their ill child. These costs were higher when compared with previous studies done during the era of monotherapy.

1112

AN EXAMINATION OF THE PHYSICAL AND SOCIAL CONSEQUENCES WOMEN WITH OBSTETRIC FISTULA EXPERIENCE IN THE DEMOCRATIC REPUBLIC OF CONGO

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Vaginal fistula is an abnormal connection between the woman's bladder and vagina (vesico-vaginal fistula) or between the vagina and rectum (recto-vaginal fistula), allowing urine or feces to leak uncontrollably. The condition most frequently occurs in women living in resource-poor countries who experience prolonged and obstructed labor. When not repaired, the condition can result in an inability to bear children. While attention has been given to traumatic fistula associated with gender-based violence in the east of the Democratic Republic of Congo (DRC), little is known about obstetric fistula and what happens to women affected. Qualitative research was conducted between March and June 2010 in 3 regions of DRC to 1) understand the characteristics of women with fistula; 2) examine physical and social consequences; and 3) compare programmatic approaches to aid women. Research methods involved key informant interviews (n = 15 participants), in-depth interviews (n = 33) and group discussions (n = 13). Women lived in remote areas, were on average under 20 years of age, had limited or no formal education, and had the condition for over 8 years. Results illustrate the extreme physical hardship and social vulnerability women face in a society where marriage and having children is critical to status and security. Women experienced physical sequelae from incontinence of urine and feces including offensive odor, sores and rashes caused by chafing, and urinary tract infections. Social consequences included marital dissolution, community rejection and ridicule, and limited economic productivity, impacting on women's mental well-being and forcing them to live an existence of isolation and shame. Results should guide policy makers in establishing treatment involving mobile surgical teams and increasing the surgical capacity of hospitals in all regions of DRC, as well as improving outreach to ensure that affected women obtain rapid treatment. Strengthening and increasing emergency obstetric services in rural areas are the best long term solutions to this devastating problem.

1113

KNOWLEDGE, ATTITUDES AND PRACTICES REGARDING MALARIA PREVENTION AND TREATMENT OF GOLD MINERS IN SURINAME

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Currently malaria transmission in Suriname is primarily related to small-scale gold mining. The knowledge, attitudes and practices (KAP) of gold miners, regarding malaria has not been studied before. To be able to design and implement effective awareness interventions, it is necessary to first assess the environment in which the implementation will take place by studying current level of KAP. In July 2009 a cross sectional study was done in three selected locations. A questionnaire was administered to miners at the Tourtonne malaria clinic (n=112), in the Tourtonne neighborhood (n=27) and in a gold mining area (n=27). 77.1% of the respondents knew that malaria is transmitted by a mosquito. Only 37.9% cited mosquito net as a means of prevention. Only 28.3% claims to sleep under a net every night. 85.5% is concerned with malaria while in the interior. 84.9% knows that malaria can be fatal. Overall 44.5% of the respondents took a malaria test the last time they thought they had malaria. Of those working in Suriname 52.0% took test vs. 46.2% working in French Guiana. Overall 55.4% engaged in self-treatment, mainly because of no or difficult access to health facilities (92.0%). Overall, 64.9% completed their last malaria treatment; however, adherence was significantly higher when prescribed by health personnel (86.0%) compared to self-treatment (48.1%), p<0.001. 38.6% of the respondents do not receive any health information. To receive malaria information 25% prefers Brazilian TV, 26.9% information sessions in gold mining areas and 15.4% from health post. Only 11.5% prefers written media. Respondents were fairly knowledgeable about malaria transmission, but demonstrated poor adherence to treatment and behavior that is not consistent with effective protection from malaria. It is important to launch interventions that focus on creating awareness on the importance of drug adherence - to reduce the emergence and spread of drug-resistant malaria - and the use of effective protective measures. The best way to reach this group is through oral and visual media.

1114

MENINGITIS IN GHANA: A SOCIO-ECONOMIC STUDY OF THE IMPACT IN THE KASSENA-NANKANA DISTRICT OF NORTHERN GHANA

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Epidemics of meningitis occur throughout the world, but the greatest burden of disease is in the "meningitis belt" of the Sahel of Africa, where widespread epidemics occur every eight to twelve years. Knowledge, Attitudes and Practices (KAP) regarding meningitis and household Cost of the Illness (COI) are poorly understood and likely to show considerable variation across the belt. A KAP and COI survey was conducted in the Kassena-Nankana (K-N) district of northern Ghana, using a case-control methodology. Quantitative interviews were conducted with 74 cases and 148 controls. The COI was computed from patients' answers to questions about direct medical costs, direct non-medical costs and productivity lost due to meningitis. Results showed that there was high knowledge about stiffness of waist or neck (68%) as a symptom of meningitis by both cases and controls, but cases were more likely than controls to mention other critical early symptoms (high body temperature OR=0.44, vomiting OR=0.35, severe headache OR=0.52, loss of appetite OR=0.20).

There were no significant differences between the cases and controls with regards to the perceived causes of meningitis: heat was the most common cause mentioned by both cases and controls (82%). The average household cost of treating meningitis was $156 per case, which is higher than the average income of farmers ($87) in the district. Much of the total cost of meningitis was from productivity lost (60%); the average number of days lost due to meningitis was 29 days. The average cost of meningitis sequelae (i.e. hearing, neurological and vision problems) was $843 per case. In conclusion, knowledge of meningitis symptoms seems to be limited to stiff neck or waist, and a greater awareness of the causes and symptoms could be achieved with more focused education. The COI survey results suggest that meningitis poses a significant burden on households through out-of-pocket payment and lost productivity. Education and vaccination against meningitis will contribute to saving lives and palliate the economic consequences of the disease.

1115

COLLABORATION WITH TRADITIONAL HEALERS TO EXPAND SURVEILLANCE FOR PLAGUE IN NORTHERN UGANDA

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Plague, caused by the bacterium *Yersinia pestis*, is an important cause of morbidity and mortality in the West Nile region of northwest Uganda. Plague needs to be treated with appropriate antibiotics to reduce mortality. Estimates for Uganda suggest that 40-60% of the population use traditional medicine (WHO 2002). In this overwhelmingly rural, upland area with limited resources, traditional healers are an important component of local health care. This may contribute to inadequate treatment and underreporting of plague cases, and occupational risk for healers. Working with staff from the Uganda Virus Research Institute and the Uganda Ministry of Health, CDC undertook a qualitative assessment with a sample of traditional healers in 2009. The goals were to learn whether healers see suspected plague patients, to understand healers' knowledge of the disease, and to assess opportunities to involve traditional healers in the referral of suspected plague patients to clinics for life-saving antibiotic treatment. Eleven healers from two districts in the West Nile region were interviewed. Healers interviewed had general awareness of plague in their area and most indicated that they see patients with symptoms that could fit the description of plague. While most reported referring suspected plague patients to the local clinic, many also described administering "first aid" for a period of hours to days before referral. There was strong willingness to participate in training about plague and to engage in referral. General characteristics of the traditional healers' practice are also described, including use of herbs and witchcraft. Based on this assessment, a pilot referral network was put into place in 2010 with 10 traditional healers. Participating healers were trained through individual visits which included local clinic staff. They were provided a bicycle, referral cards and a cell phone programmed with minutes and clinic contacts. Initial results of this referral program will be presented.

1116

ACCEPTABILITY AND FEASIBILITY OF ELECTRONIC INFECTIOUS DISEASE SURVEILLANCE USING MOBILE PHONE TECHNOLOGY

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In India, physicians and other health-care personnel are increasingly using mobile phones and computers for daily communication and information management. However, application of these technologies to public health surveillance in India has been limited. We hypothesized that mobile-phone based electronic surveillance system will provide an acceptable alternative to standard, paper-based data collection forms that are typically used for collection of surveillance data in India. Rotavirus surveillance was established using a paper- and phone-based surveillance system for rotavirus diarrhea in and around Kolenchery, Kerala, India. We conducted a pilot study to evaluate the feasibility of implementing a mobile phone-based data entry system for rotavirus surveillance data collection. We surveyed current- and potential-users of the phone-based system using a structured questionnaire. A total of 186 hospital staff completed the survey including nine current and 177 potential users including nurses, administrative workers and physicians from eight hospitals. The mean age of current- and potential-users was 38.2 and 29.0 years, respectively. A total of 126 (68%) were physicians or nurses. Eighty-eight percent of respondents were willing to use the phone-based data collection system on a daily basis. The two most commonly cited concerns for regular use of the PDA in surveillance data collection were accuracy (34% potential *vs* 56% current users) and confidentiality of collected information. The mobile phones were capable of accurately displaying the data collection form and allowed for touch screen data entry and data storage using removable memory devices. In conclusion, our results suggest that mobile phone-based electronic surveillance data collection systems are acceptable and feasible for rotavirus surveillance in India. Scale-up and further evaluation of the phone-based system for surveillance data collection will now be developed in conjunction with local physician groups in India.

1117

THE IMPORTANCE OF AN INTEGRATED RESPONSE TO CHOLERA PREVENTION AND TREATMENT: PSYCHOSOCIAL SUPPORT TO SURVIVORS OF CHOLERA

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Along with the devastation the recent cholera outbreak in Haiti has brought, adding insult to injury to an already struggling country, has come stigma and anxiety around the origins of the disease and how it is transmitted. The sudden loss of life associated with cholera, and the inability to perform proper burials for those who have died, has brought back vivid memories of the tragic earthquake of January 2010, thus negatively impacting one's mental health. With a majority of Haitians living in areas without improved water sources and far from health centers, more than 5000 people have died and thousands more have been infected with the deadly bacteria. In mid-October 2010, Partners In Health/Zanmi Lasante (PIH/ZL), found ourselves in the midst of the epicenter of the outbreak, being the major partner working with the Haitian Ministry of Health's public hospitals along the Artibonite River valley. New to treating cholera, PIH/ZL quickly sprang into action, leaning on our multidisciplinary community-based model used for treating other diseases to guide in developing our rapid prevention and treatment response. As more people die and stigma against people with cholera

continues to rise, the psychosocial support team has developed a series of memorial services and support groups for families and individuals coping with the impact cholera has had on their lives. ZL psychologists have been leading memorial services to help with the healing process, but also to aid in reducing stigma. In the same vain, ZL developed support groups for survivors of cholera to help them regain their positive body image and aid in reintegration into their families and communities. Support group participants have noted a positive effect on their lives, giving them a shared space to discuss their experiences and support each other through their healing process. As the epidemic continues, the support groups and memorial services remain integral to the community response, working with traditional healers and community leaders to dispel myths surrounding the origins and spread of cholera.

1118

IN VITRO INVESTIGATION OF *PHYLLANTHUS FRATERNUS* AS AN ANTI-INFECTIOUS MEDICINAL PLANT

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In the last few years, a number of studies have been conducted in different countries to prove the antimicrobial properties of medicinal plants with high efficiency. It is in the spirit of continuing herbal medicine research that, the antimicrobial activity of aqueous extract from *Phyllanthus fraternus* was evaluated against seven standard and fourteen clinically important isolates using the agar-well diffusion method. In addition, the possible *in vivo* toxic effects from the extracts as well as the presence of phytochemicals were studied. Extracts from *P. fraternus* inhibited the growth of 5 out of 7 (71.4%) standard strains with zones of inhibition ranging from 0.0 to 29.67 ± 0.33mm while in the case of the wild strains, growth of 7 out of 14 (50%) strains were inhibited with zones of inhibition ranging from 0.0 to 14.33 ± 0.33mm. It was also observed that all the Gram positive bacteria (100%) were inhibited by *P. fraternus* whilst only 5 out of 15 (33%) Gram negative bacteria were inhibited. Thus, the growth of a total of 12 out of 21 (57%) microbes used were inhibited by the extract from *P. fraternus* with an average zone of inhibition of 9.37 ± 2.17mm. Significant phytochemicals detected were phenolics, polyuronides, reducing sugars, triterpenes and alkaloids. The LD_{50} value was found to be greater than 5000 mg/kg making P *P. fraternus* practically non-toxic according to Hodge and Sterner Scale.

1119

EVALUATING THE COST-EFFECTIVENESS OF CHECKLISTS AND TREATMENT ALGORITHMS: AN EMPIRIC EXAMPLE OF A MENINGITIS CHECKLIST IN RESOURCE LIMITED SETTINGS

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Checklists can standardize patient care, reduce errors, and improve health outcomes. In meningitis, with high patient loads, limited financial resources, and high mortality, CNS diagnostic algorithms may be a useful tool to guide diagnosis and treatment in resource limited settings. However, the cost-effectiveness of such algorithms is unknown. We developed a decision analysis model to evaluate 3 diagnostic strategies to assess the costs, diagnostic yield, and cost-effectiveness for CNS infections. Strategies were: 1) comprehensive "shotgun" approach of all available testing; 2) stepwise strategy with testing in a specific order; 3) minimalist strategy of high-yield testing only. Each strategy resulted in 1 of 4 meningitis diagnoses: bacterial, cryptococcal, TB, or other (aseptic) meningitis. In model development, we utilized published prevalence data from Cape Town, South Africa and published diagnostic test performance. We validated the 3 strategies in a prospective Ugandan cohort. The current comprehensive strategy resulted in 97% of patients with correct diagnoses at an average cost of $38.08/patient. The stepwise strategy had 93% correct diagnoses costing $15.74/patient, and minimalist strategy had 91% correct diagnoses costing $9.96/patient. The incremental cost effectiveness ratio was $308.03 per additional correct diagnosis for the stepwise over the minimalist strategy and $519.88 for the comprehensive over the stepwise strategy. As the proportion of negative lumbar punctures reached 50% (i.e. no meningitis), the costs increased to $21.32 per patient in the minimalist strategy; $30.49 in the stepwise strategy and $78.60 in the comprehensive strategy. Designing checklists and algorithms with consideration of both "effectiveness" and "costs" is essential. Through strategically choosing the order and type of testing coupled with disease prevalence rates and local medical practice, algorithms can be cost-effective and potentially sustainable in resource limited settings.

1120

PARTNERSHIPS IN FACILITY AND COMMUNITY-BASED RESPONSE TO A CHOLERA EPIDEMIC IN HAITI

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Partners In Health (PIH) supports the Haitian Ministry of Health (MOH) to provide comprehensive health care to the poor. In October 2010 cases of acute fatal watery diarrhea amongst adults in St Marc, Haiti signaled the beginning of a cholera epidemic. In its role supporting 12 medical facilities in Haiti, PIH was involved from the beginning of the epidemic and continues to see an average of 5000 patients per month (April 2011). Two major challenges to the initial crisis were staffing and materials supply chain management. In both cases, partnerships were critical in saving lives in the first days. As cholera had not been previously reported in Haiti, existing healthcare providers had no experience with the rapid fatal nature of the disease and were quickly overwhelmed by volume of patients and severity of illness. Rapid reinforcement of clinical teams with PIH staff from other locations in Haiti provided initial backup but was soon exhausted as the epidemic spread to other regions and staff members were needed at their home base. PIH's partners program in Global Health Equity at Brigham and Women's Hospital, Boston USA sent experienced medical residents for shift work. Technical assistance, particularly in establishing infection control measures was essential for rapid knowledge transfer on best practice and was provided by a partner NGO with cholera experience. Materials and supplies were rapidly consumed. An existing partnership resulted in one NGO in an unaffected area sending materials, supplies and staff within hours of the outbreak without delays of formality. Collaboration with new NGOs in a high-stress environment during a major crisis posed many challenges such as: differing compensation schemes, differing organizational cultures and language skills, duplication of reporting and some degree of competition for resources. Despite these challenges, partnerships were essential to success and should be encouraged by donors, governments and NGOs and established before disasters occur so that response is more efficient during times of crises.

1121

QUALITY OF LIFE IN FILARIAL LYMPHOEDEMA PATIENTS IN COLOMBO, SRI LANKA

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Lymphatic filariasis (LF) is an important global public health and socio-economic problem. It affects 120 million people in over 80 countries, of which, about 14 million suffer from lymphoedema or elephantiasis of legs. Although LF does not cause immediate mortality, the associated severe morbidity has resulted in it being recognized as the second

leading cause of disability worldwide. The two most common chronic manifestations of the disease - hydrocoele and lymphoedema, cause socio-psychological problems to patients and their families. Chronic disease is debilitating, leading to a restriction in the duration and capacity to work and to changes in activity patterns. Secondary bacterial infections of lymphoedematous limbs, known as acute adenolymphangitis (ADL) attacks, contribute to the morbidity of patients as well as progression of the lymphoedema. The quality of life (QOL) was assessed in 141 filarial lymphoedema patients and 128 healthy individuals in the Colombo district of Sri Lanka. Information was gathered by administering the validated translated version of the WHO 100-item QOL questionnaire (WHOQOL-100). This questionnaire ascertains an individual's perception of QOL in the physical, psychological, level of independence, environmental and spiritual domains, as well as the general QOL. There is no documentation of the WHOQOL-100 having been used in filarial lymphoedema patients prior to this study. Healthy controls had a better QOL in all domains as well as in the overall general QOL, when compared to patients with lymphoedema. Several facets such as pain and discomfort, sleep and rest, activities of daily living, dependence on medication and treatment, working capacity and social support were significantly affected by the ADL attack/s patients had suffered. The environmental and spiritual domains were significantly affected by the maximum grade of lymphoedema. The significant difference in the QOL as perceived by patients suffering from filarial lymphoedema and apparently healthy individuals reiterates the importance of morbidity control in patients already affected by filarial lymphoedema.

1122

IMPACT OF A COMMUNITY-BASED LYMPHEDEMA MANAGEMENT PROGRAM ON EPISODES OF ADENOLYMPHANGITIS (ADLA) - ORISSA STATE, INDIA

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India has an estimated 7 million people with lymphedema due to lymphatic filariasis (LF). Clinic-based programs to promote lymphedema management at home have been shown to decrease episodes of adenolymphangitis (ADLA), but the effectiveness of community-based programs, which can potentially achieve higher coverage at lower cost, has not previously been studied. A community-based lymphedema management program was implemented in Orissa State, India in 2007 by the Indian non-governmental organization, Church's Auxiliary for Social Action, in consultation with the Centers for Disease Control and Prevention. Health workers teach lymphedema management techniques to >20,000 lymphedema patients. All 330 lymphedema patients in 20 randomly-chosen villages in areas without a previous lymphedema management program and 45 patients with advanced lymphedema in neighboring villages also without a current program were selected for the study, for a total of 375. Patients were followed over 12 months to evaluate the program's impact. Data were collected at baseline and at 1, 2, 3, 6, and 12 months and analyzed using longitudinal analysis procedures. At baseline, the rate of ADLA episodes per person-month was 0.35 compared to 0.14 at 6 months ($p<0.0001$) and 0.23 at 12 months ($p=0.0047$). The rate ratio (RR) of ADLA episodes per person- month among patients at 6 months compared to baseline was 0.40 (95% CI: 0.50, 0.88) and at 12 months was 0.66 (95% CI: 0.50, 0.88). Significant differences were also seen in the rates at 1 month and 3 months when compared to baseline. A marginal Poisson model showed that the rate of ADLA episodes decreased among patients who wore footwear outdoors (RR=0.66, 95% CI: 0.48, 0.92) and increased among patients who used anti-fungal cream (RR=1.81, 95% CI: 1.15, 2.84). Interdigital fungal infections are a risk factor for ADLA and the increased risk of ADLA episodes among patients using anti-fungal cream is consistent with cream being a marker for this risk factor. These data show a beneficial impact of the program at 12 months, but evaluation at later time points is needed.

1123

FLUBENDAZOLE AS A POTENTIAL MACROFILARIACIDE FOR FIELD USE

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A safe, field-usable chemotherapeutic agent that will rapidly kill adult filarial worms is urgently needed in tropical medicine. Ivermectin, distributed as Mectizan® by Merck & Co.Inc. has had an enormous impact on two major human filarial infections of developing countries, onchocerciasis and lymphatic filariasis. However, a macrofilaricide that safely kills adult filarial worms would be a major contributor to the current efforts to rid the world of filarial infections and the diseases they cause. Given the challenges of discovery and development of agents for human use, a drug as described above is arguably most likely, at least at present, to come from the benzimidazole group of anthelmintics. A field useful agent has typically been required to be administered in an oral dosage form, but a truly safe agent administered by another route, including parenteral approaches, could be acceptable and may even be advantageous. We believe that the most appealing benzimidazole with regard to filarial parasites is flubendazole as it is highly active against filariae in a number of hosts. It has the typical benzimidazole structure with an added fluorine as the major structural difference from other benzimidazoles. Flubendazole is highly efficacious in various experimental filariasis models, including the feline *Brugia pahangi* model, a host in which it occurs naturally. This presentation will review the available data on the use of flubendazole in treating infections with filariae and tissue-residing helminths and describe the characteristics needed for reformulating this important macrofilariacide for potential human use.

1124

REINVIGORATION OF LYMPHATIC FILARIASIS MORBIDITY PROGRAM FOR THE GLOBAL PROGRAM

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The Global Program for the Elimination of Lymphatic Filariasis has two major arms to the efforts, the distribution of anti-microfilarial drugs to break transmission and the morbidity management and disability preventions activities for those already affected by the disease. Despite the good efforts of many institutions and field-working groups, the latter of these two components has often lagged behind the efforts and attention paid to distribution of drugs to the eligible population; this has occurred for many reasons that include poor funding, a general lack of knowledge of the prevalence, and confused understanding of the optimal approaches to treating and assisting these unfortunate people. The actual number of those affected is not known in many countries. There are many reasons why there is great value, in addition to the obvious humanitarian need, in attending to the needs of lymphatic filariasis patients. Attending to patients has a positive effect on improving coverage, and the improvements in patients that has been seen as a result of implementing mass drug administration programs in a number of countries has contributed to improving drug coverage in the whole population. It is vital,with many countries beginning to wind down their drug distribution

programs as infection levels dramatically reduced, that efforts made to attend to those who will still be suffering from the clinical consequences of the infection which extend long after infection and transmission has ceased. These affected people are in danger of being again forgotten when MDA programs cease with elimination. This presentation will review the renewed efforts currently being made to enhance and widen the morbidity management and disability prevention efforts in endemic countries.

1125

THE INTERACTION OF THE INFECTIVE STAGE OF *BRUGIA MALAYI* WITH LANGERHANS' CELLS

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Filarial infections are initiated by deposition of the infective larvae (L3) in the skin, a process that likely conditions the priming of the immune system to the parasite. Thus, understanding the interaction between the L3 and the antigen presenting cells in the skin becomes crucial. Previously, by using ex-vivo epidermal skin explants exposed to L3 in contact or in transwell, it was shown that the expression of IL-18 protein and of mRNA for caspase 1, CD207 (Langerin), and IL-18 binding protein (BP) was induced by the L3. Since caspase 1 is central to the inflammasome, we further investigated the potential involvement of inflammasomes in L3-exposed LCs. We generated human LCs (Langerin+, E-cadherin+, CD1a+) *in vitro* using conventional techniques and exposed them to either L3 (in contact or in transwell), LPS, or media and assessed their expression of cell surface markers, production of pro-inflammatory cytokines and expression of the genes involved in inflammasome activation. In contrast to a known activator of the inflammasome, LPS, L3s only induced minimal up-regulation of surface expressed CD14 (with concomitant down-regulation of CD1a), CD86 and CD83 with no changes in surfaced expressed CD207, E-cadherin, CD80, CD40 and HLA-DR. No significant changes in mRNA expression were seen between LC and LC exposed to L3 for the inflammasome-associated genes NLRPs, NLPR1, NLRC4, AIM2, ASC and IL-18, although there was increased (but not statistically significant) expression of I L-18BP and caspase 1. L3 failed to induce the production of the cytokines IL-1β, IL-6, IL-8, IL-18, IL-18BP, IL-33 and IFN-γ from *in vitro* LCs, nor did the L3 condition the LC to suppress proinflammatory responses to LPS or Poly I:C. The apparent discrepancy between L3 exposed skin explants and the *in vitro* generated LCs can be explained by the presence of keratinocytes (KC) in the ex vivo model; the role of KC/LC interaction in the context of L3 exposure is ongoing.

1126

LOA LOA INFECTIONS AT A TERTIARY REFERRAL CENTER: REFINING THE CLINICAL AND IMMUNOLOGICALLY BASED DIFFERENCES BETWEEN TEMPORARY RESIDENTS AND THOSE INDIGENOUS TO LOA-ENDEMIC AREAS

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Previous studies have suggested that Loa loa infections in inhabitants of Loa-endemic areas (END) have marked differences in clinical presentation compared to those of temporary residents (TR). Many of these differences are thought to be immune-mediated. To assess these differences in clinical outcome and pathogenesis of Loa loa infection, we conducted a retrospective analysis of 181 patients with loiasis seen at the National Institutes of Health. Among the 181, 37 were raised in L. loa-endemic regions while 144 were visitors to these same regions. The initial clinical presentation differed markedly between the two groups with only 41% of END having a history of Calabar swelling compared to 79 % of TR (p<.001). In contrast, the END were much more likely to have had eyeworm (62% compared to 14%, p<.001) and were more likely to have microfilaremia (73% compared to 23%, p<.001). TR were more likely to have an atypical presentation of infection including urticaria (19.4% vs 2.7%; p<.05). There were no differences between the groups in gastrointestinal symptoms (p=.118), neurologic symptoms (p=.29), rashes (p=.33), pruritus (p=.83), or cardiomyopathy (p=.81). Although there was not a statistically significant difference in the serum levels of polyclonal IgE between the two groups (geometric mean [GM] 883 IU/mL in END vs 294.7 IU/mL in TR, p=.651), the serum levels of polyclonal IgG differed significantly (GM 1538 IU/mL in END vs 1168 IU/mL in TR, p<.01). There was no significant difference in filarial (BmA)-specific IgG or IgG4 between the two groups, (BmA-specific IgG 696 mg/mL IgG in END vs 458 mg/mL in TR; BmA-specific IgG4 295 ug/mL END vs 89 ug/mL in TR). Most notably, the absolute eosinophil counts (AEC) were markedly different between the groups; the GM AEC in TR more than two-fold higher (1505/uL compared to 730/uL) than in the END (p=.034). These data extend earlier observations related to immunologically based clinical differences between TR and END. Additional data concerning eosinophil-related pathogenesis of loiasis will be discussed.

1127

VACCINATION OF BALB/C MICE WITH INTESTINAL ANTIGEN FROM *LITOMOSOIDES SIGMODONTIS* FAILS TO PROTECT AGAINST CHALLENGE INFECTION

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Intestinal antigens have shown promise as vaccine candidates in a number of helminth models. In this study, we evaluated the immune responses that develop towards filarial intestinal antigens in mice infected with *Litomosoides sigmodontis*, a murine model of filariasis in which infective-stage L3 larvae develop into mature adult worms in immunocompetent Balb/c mice. A crude homogenate of soluble intestinal antigens from *L. sigmodontis* worms (GutAg) was prepared from intestinal tracts obtained from adult female worms by microdissection. At both 8 and 16 weeks after infection, splenocyte proliferative responses towards GutAg were substantially lower than that which developed towards a crude homogenate of whole worm antigen (LsAg). Similarly, IgG antibody titers and splenocyte production of both IL-4 and IFNγ were lower in response to GutAg than LsAg at all timepoints studied. Vaccination of mice with three weekly intraperitoneal injections of 10 micrograms of GutAg with CPG and alum as adjuvant resulted in marked splenoctye proliferation and IL-4 and IFNγ production in response to GutAg as well as titers of GutAg-specific IgG antibodies measurable at dilutions up to 1:175. Despite the induction of GutAg-specific immune responses, an initial challenge experiment demonstrated no protection in GutAg vaccinated mice as compared to controls. These preliminary results suggest that filarial infections do not induce large immune responses to intestinal antigens and that inducing such responses may not be protective. Further trials are underway to determine whether vaccination strategies that induce greater antibody titers can be protective.

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ENDOTHELIAL CELLS RELEASE SOLUBLE FACTORS THAT PROLONG THE SURVIVAL OF *LITOMOSOIDES SIGMODONTIS* MICROFILARIAE *IN VITRO*

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Microfilariae of most filarial pathogens typically survive for months in their vertebrate hosts. *In vitro*, however, microfilariae live for much shorter time periods. To begin characterizing the factors microfilariae require for

prolonged survival, we have conducted a series of *in vitro* experiments using microfilariae obtained from gerbils infected with Litomosoides sigmodontis, a filarial parasite of rodents. While culture of microfilariae in Dulbecco's Modified Eagle Medium supplemented with 10% FBS resulted in average survival of only 7 days, co-culture of microfilariae with a mouse endothelial cell line (EOMA) extended survival to 40 days. Not all cell lines have this property, as all microfilariae co-cultured with a mouse myeloma cell line died by day 10. Co-culture experiments using EOMA cells in transwell plates extended microfilaria survival as well as direct co-culture, suggesting that the factors microfilariae require are soluble in nature. Heat inactivation of conditioned media from EOMA cells resulted in average microfilaria survival of only 3 days. Together, these findings demonstrate that microfilariae require heat-stable factors released from endothelial cells for prolonged survival. By identifying a cell line that does not promote microfilaria survival, we are poised to begin biochemical and comparative analyses to elucidate the chemical nature of these essential factors. Identification of such factors will advance our ability to cultivate filarial pathogens *in vitro* and may provide insights for the development of new antifilarial compounds.

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ENTOMOLOGICAL STUDIES TO RE-EXAMINE THE EVIDENCE FOR MASS DRUG ADMINISTRATION FOR FILARIASIS ELIMINATION IN WEST AFRICAN CAPITALS: THE CASE FOR FREETOWN, SIERRA LEONE

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Anopheles mosquitoes are the principal vectors of lymphatic filariasis in West Africa where the urban mosquito, *Culex quinquefasciatus*, is less susceptible to *Wuchereria bancrofti* found in the sub-region. The transmission of lymphatic filariasis (LF) in West Africa is mostly rural, with little or no evidence of active transmission in the capital cities of Accra, Conakry and Freetown. Night blood surveys carried out on 500 adults in the Western Urban District of Freetown in 2007 revealed no MF positive individuals. However, the district comprising of greater Freetown was considered endemic for LF based on antigen positivity using ICT cards. During the civil war, a large population of Internally Displaced Persons (IDP) moved to Freetown from the rural areas where LF is endemic. A study conducted among these IDPs in 1997 revealed an antigen prevalence rate of 14.5%. Based on the presence of antigen positive individuals in Freetown, MDA was carried out in the capital in 2010. The present study tests the hypothesis that populations of limited low density microfilaremia carriers settling in urban cities in West Africa are incapable of initiating LF transmission by the less efficient *Anopheles* mosquito species. The second objective of the study was to determine the role of *Culex quinquefasciatus* in LF transmission in Freetown. Mosquitoes were collected, using the pyrethrum spray sheet method, from the communities were ICT positive individuals were found. Since May 2009, a total of 6327 *Cx. quinquefasciatus,* 603 *Anopheles* and 6 *Aedes* mosquitoes have been tested by PCR and none was found positive for *W. bancrofti*. These results suggest no evidence for ongoing transmission, and the current MDA campaign in Freetown may not be necessary. This study makes a case for the need to determine active disease transmission for targeted resource utilization, especially since the LF elimination program requires treatment for endemic communities for at least 5 years.

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THE CHANGING STATUS OF FILARIASIS IN TANZANIA

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Tanzania has been one of the Africa's leading countries in the global effort to eliminate lymphatic filariasis in Africa. Historically Tanzania has been involved in research for over half a century, and was one of the first countries in Africa to begin the mass drug administration (MDA) of ivermectin and albendazole to eliminate this affliction from the country. The coastal regions of Tanzania has been known for many years to be site were some of the worst prevalences of clinical filariasis in Africa are found with up to 15 percent of adults affected in some way with over clinical signs and symptoms of filariasis. The extent of filariasis in the country was assessed in 1999-2000 as a essential step in developing the national MDA program using rapid antigenaemia tests (ICT) and clinical data of the presence of the disease in each political district in the country. At this time is was found, contrary to what was expected at that time, in fact the whole country was eligible for the initiation of the MDA program for filariasis; thus the target population for the national program was around 35 million people. The MDA program began with a scaling up of new areas each year to reach some 15 million treatments in 2008. Assessment of various districts after 5 and 7 years for MDA has shown that area which were around 70% ICT positive have now been reduced to less than 5% and to around 1% in a number of areas. Analysis of the reduction in prevalence of ICT and circulating microfilarial loads suggests that it is important to consider prevalence levels (e.g. hyper-endemicity versus hypo-endemicity) when considering expectation for the length of time of program implementation, the needs for morbidity control; activities to enhance MDA activities, etc. Data from the archipelago island district of Mafia, with its very heterogenous population (farmers and fishermen) clearly show that adaptation of MDA to suit the local community is essential to obtaining successful reduction on prevalence of the parasite. Tanzania has seen a remarkable change in parasite prevalence and clinical disease as a direct result of the MDA programs.

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QUANTIFYING THE ECONOMIC BENEFITS OF A COMMUNITY-BASED LYMPHEDEMA MANAGEMENT PROGRAM - ORISSA STATE, INDIA

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There are an estimated 59 million people with lymphatic filariasis (LF) in India; an estimated 19.6 million of whom have chronic filarial disease. Orissa State is highly endemic for lymphatic filariasis with many people requiring lymphedema care for lymphedema or elephantiasis. Nevertheless, there are little data on the cost associated with scaling up lymphedema management programs in LF endemic countries. A community-based lymphedema management program was implemented in Orissa State by the Indian non-governmental organization, Church's Auxiliary for Social Action, in consultation with the Centers for Disease Control and Prevention. Over a three year period from 2007-2010, 21,468 lymphedema patients were sequentially recruited into the program in Khurda district, an LF endemic district in Orissa State; 5,478 patients enrolled between 2007-2008, 9,996 patients enrolled between 2008-2009, and 5,994 patients enrolled between 2009-2010. Activities of the program entailed: health education on lymphatic filariasis prevention, disease, and clinical management; lymphedema management training for medical and paramedical staff and community health workers; and support to the lymphedema patients, including provision of soap, antifungal ointment, towels and in some cases footwear. The start-up

cost per patient varied from US $6.75-$9.00 and the maintenance cost per patient was US $3.50. The majority of the total program cost (64%) went to providing direct care (training, follow-up and supplies) for the lymphedema patients. At 12 months after enrollment, patients reported a total of 28.8 (range, 15.6-38.4) fewer lost days of productivity than prior to enrollment in the lymphedema management program. Extrapolated over the entire enrolled population (n=21,468) translates into greater than 1600 person years of productive labor saved over the first year of the program. Despite higher start-up costs, community-based lymphedema management programs can have a broad beneficial impact by improving patient productivity.

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SCREENING FOR NOVEL ANTHELMINTICS THAT ACT ON NEMATODE NEUROPEPTIDE RECEPTORS

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Neuropeptides in the FMRFamide family (FLPs) are essential components of nematode neuromuscular systems and regulate essentially all physiological systems involved in motility, eating and reproduction. A large family of G protein-coupled receptors (GPCRs) that employ FLPs as endogenous ligands have been identified from the free-living nematode Caenorhabditis elegans as well as from many parasitic species. cDNAs encoding ~ 10 FLP-GPCRs have been functionally expressed in yeast (Saccharomyces cerevisiae) in a format that allows facile, multiplexed high-throughput screening assays to identify small molecule, non-peptide ligands that act as agonists or antagonists of these GPCRS. These assays are based on ligand-induced receptor activation, which leads to expression of an enzyme in the histidine biosynthesis pathway that is otherwise absent from this strain of yeast. The presence of an agonist in the culture medium thus permits growth of the recombinant yeast in the absence of histidine, providing a sensitive and highly specific endpoint for screening. Non-peptide agonists and antagonists of nematode FLP receptors are intriguing leads for possible development as novel anthelmintics. We adapted and optimized 3 recombinant yeast strains for screening a collection of synthetic and natural product chemicals held at McGill (HTS/HCS facility, Department of Biochemistry) as part of the Canadian Chemical Biology Network, and for screening collections of diverse natural products and synthetic chemicals in Cape Town and Gaborone. We present here a description of the screening system and results from the initial screening assays of natural products held in collections on both continents.

1133

ANALYSIS OF ß TUBULIN GENE SEQUENCES OF *NECATOR AMERICANUS* IN AREAS OF GHANA WITH LONG-TERM EXPOSURE TO IVERMECTIN FOR ONCHOCERCIASIS CONTROL

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In Kintampo North District (KND), Ghana, ivermectin (macrocyclic lactone) has been used in Mass Drug Administration for Onchocerciasis control since 1980s and recent studies reported albendazole (benzimidazole) failure rates of 39-54% in hookworm treatment. Unlike for human health, benzimidazole (BZ) resistance in the veterinary field is well documented, and is associated with single nucleotide mutations in the β-tubulin isotype-1 gene that result in amino acid changes Phe167Tyr, Glu198Ala and Phe200Tyr in the gene product. Furthermore, it has been reported that the use of macrocyclic lactone could result in such changes. We therefore investigated the sequences of β-tubulin gene for potential BZ resistance marker in Necator americanus obtained from stool specimens of 210 surveyed individuals in rural communities in KND. The prevalence and intensity of hookworm infection was determined using the Kato-Katz method. Hookworm eggs were cultured by a modified Baermann method and DNA extracted from larvae using a modified proteinase K method. Primers for a nested PCR method were designed from published β-tubulin sequences and used together with a proof-reading polymerase to amplify these regions of interest. Eight PCR products were sequenced and aligned using Multalin™. These were translated to amino acids using Expasy™ (Swiss Institute of Bioinformatics). Results indicated egg intensity range of 2688epg - 24epg. *Necator americanus* was the most abundant hookworm with a prevalence of 65% (48/74) while *Ancylostoma duodenale* prevalence was 26.7 % (25/74). Analysis showed several single nuclotide mutations. Nine amino acid changes namely; Phe167Val, Asp197Met, Glu198Lys, Glu198Arg, Thr199Pro, Phe200Ser, Cys201Val, Asp203Ile and Asn204Ile were considered potential resistance markers on the basis that substitution by an amino acid of different properties was likely to affect BZ metabolism. This preliminary study has for the first time, to our knowledge, revealed amino acid changes in the N. americanus β-tubulin gene and we intend to conduct further epidemiologic studies to hone in definitive biomarkers of BZ resistance.

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PARASITIC ZOONOSES CHILDREN UNDER SEVEN YEARS ASSOCIATED WITH THE COEXISTENCE WITH DOMESTIC DOGS

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The objective of the present study was to determine the prevalence of zoonotic parasitic infections in children under seven years, associated with the coexistence with domestic dogs belonging to a village of the township of "Los Garzones" the city of Monteria, Cordoba 2010. The study was descriptive cross-sectional. The sample consisted in 71 families which were selected infant population of 42 children under seven years and all the dogs that lived with the children. After family motivation on work goals and ensure informed consent, we proceeded to the collection of fecal samples in children and dogs. The samples were processed in the Microbiology laboratory of the Faculty of Health Sciences, Department of Bacteriology, University of Córdoba by: fresh preparation, techniques Ritchie method by centrifugation and flotation method, modified Ziehl-Neelsen stain and Graham method. The prevalence of parasitic infections in humans highlighted by *Ascaris lumbricoides* (38.10%), *Strongyloides* spp. (28.57%), hookworm (21.43%). *Entamoeba coli* (33.33%), *Giardia lamblia* (26.19%) and *Cryptosporidium* spp (14.29%). In dogs parasitic infections were found to *Strongyloides* spp. (50.00%), Genera *Ascaris* and *Toxocara*, *Toxascaris* (38.10%), hookworm (33.33%). *Entamoeba coli* (40.48%), *G. lamblia* (26.19%) and *Cryptosporidium* spp (30.85%). In conclusion, the investigation could conclude that in the studied community there are predisposing factors for the submission of parasitic diseases in humans and dogs. The finding of common parasites in children and animals suggest that living with animals is a risk factor for transmission of parasitic infections.

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IDENTIFICATION OF IMMUNDOMINANT *TOXOCARA* EXCRETORY-SECRETORY ANTIGENS

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Toxocariasis is the infection of a human host caused by ingestion of embryonated eggs of the canine or feline roundworm, *Toxocara canis* or *T. cati,* and the subsequent invasion of body tissues by migrating L3 larvae. Infections are divided into 2 major syndromes, visceral toxocariasis and

ocular toxocariasis, both of which can result in extensive tissue damage and, on rare occasions, can lead to eosinophilic meningitis when larvae enter the CNS. Due to widespread endemicity and the risk of morbidity, it is important to have a sensitive and specific assay for diagnosing human toxocariasis. Current diagnosis is dependent on an Enzyme-linked Immunosorbent Assay (EIA) using crude antigen. To develop an improved diagnostic assay for toxocariasis, our aim was to first identify immunoreactive proteins in the *T. canis* excretory-secretory (TES) products from *in vitro* cultivated L3 larvae. Separation of the L3 TES proteins was performed with two-dimensional gel electrophoresis (2DE) on 4-12% Bis-Tris gels. Of three identical 2DE gels, two were transferred to nitrocellulose membranes and probed with either a serum pool prepared from *Toxocara* infected persons or a normal human serum sample; the third gel was stained using a mass spectrometry (MS) compatible silver staining method. Spots showing reactivity when probed with positive sera by Western blot were excised from the silver stained gel for MS analysis. A MASCOT search of the NCBI database identified only 2 *T. canis* proteins from 24 reactive spots suggesting that further studies are needed to define the proteins which could not be identified using existing databases. Choosing the ideal diagnostic protein(s) may require evaluation of up to 24 different antigenic protein targets.

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DIAGNOSIS OF INTESTINAL PARASITIC INFECTIONS USING FLUORESCENCE MICROSCOPY IN CAMEROON

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Intestinal parasites are a real public health problem in developing countries. They are generally responsible for many symptoms among which malabsorption, anemia, abdominal pain. Diagnostic methods based on microscopic identification of parasites remain common in developing countries, despite their low sensitivity. Recently, new fluorescent microscopes with light emitting diodes have improved the diagnosis of other protozoan parasites such as malaria using a DNA-specific dye DAPI (4',6-diamidino-2-phenylindole. This study was designed to compare a rapid fluorescence microscopy - based method for diagnosis of intestinal parasites to classical microscopy and to collect epidemiological data in rural and urban settings to Cameroon. From september 2009 to march 2010, 583 stool samples from outclinic patients were analyzed, including 300 in the city of Douala and 283 in the rural area of Njombe. Each sample was submitted to direct microscopic examination and formalin-ether concentration technique. The observation under fluorescence and white light was made using a fluorescence microscope CyScope® (Partec GmbH, Görlitz, Germany). Stool samples had less visible artifacts under fluorescence and helminth eggs were very clearly observed. In opposite, protozoa were better distinguished using white light. The search for parasites was positive in 155 (26.6%) of the 583 patients in the study. The prevalence in Njombe was significantly higher than Douala (39.2% against 14.7%, P <0.001). The most common prevalent species in Douala was *Entamoeba histolytica* (10.3%), while in Njombe, *Schistosoma mansoni* dominated 13.1%. This work has confirmed a high prevalence of intestinal parasites in a rural area of Cameroon and has also shown that the simultaneous use of white and fluorescence lights for stool exams could help to better observe parasites. Thus, the use of fluorescence microscopy for routine diagnosis of intestinal parasites deserves further investigation.

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MOLECULAR EPIDEMIOLOGY OF ASCARIASIS

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More than 1 billion people are infected with the giant intestinal roundworm, *Ascaris*. Although the greatest numbers of infected individuals are found in Asia and sub-Saharan Africa, ascariasis shows a cosmopolitan distribution and cases are found in both developing and developed countries. We are using molecular epidemiology techniques to study the population structure of *Ascaris* at a global and local scale. Around 550 ascarid worms were obtained from human and pig hosts in East Africa, Asia and Europe. Genomic DNA was extracted from all worms and a 383 base pair region of the mitochondrial cytochrome c oxidase 1 gene (cox1) was sequenced for each worm. Sequences were aligned to identify substitutions, and phylogenetic analysis and assessment of genetic diversity was undertaken. Microsatellite analysis of the *Ascaris* DNA is also underway. Over 70 different cox1 barcodes have been identified in *Ascaris* from humans and pigs so far. There is near complete segregation of barcodes between pig and human worms in Africa but in Europe the same barcodes are found in worms from both hosts. Further analysis should provide insights into the transmission dynamics of *Ascaris* in developed and developing countries.

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STRONGYLOIDES STERCORALIS: METHODS OF DETECTION AND EFFICACY OF TREATMENT IN SCHOOLCHILDREN IN CAMBODIA

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Worldwide, 30-100 millions people are infected with *Strongyloides stercoralis*, one of the most neglected soil-transmitted helminth (STH). Detailed information on the parasite is scare and diagnosis poses a problem. Our study aimed to compare two different diagnostic methods (Koga agar and Baermann technique) for *S. stercoralis* infection in a multiple stool examination approach and to assess the efficacy of ivermectin treatment. We performed a cross-sectional study on *S. stercoralis* infection and STH in 458 children from four primary schools in semi-rural villages close to Phnom Penh by using different diagnostic procedures (Kato-Katz, Koga Agar and Baermann technique) on 3 stool samples. Infected children were treated with ivermectin (200mcg/kg PO, over 2 days) and were reexamined 3 weeks after treatment. Hookworms, *S. stercoralis, Trichuris trichiura* and small trematode eggs (STE) were frequently observed. 24.4% of children were infected with *S. stercoralis*. The sensitivity of Koga-Agar technique and Baermann method was 88.4% and 75.0%, respectively. The negative predictive value of Koga-agar and Baermann was 96.4% and 92.5%, respectively. The cumulative prevalence of *S. stercoralis* was considerably increased from 18.6% to 24.4 after analyzing 3 stool samples by either employed methods, which was much close to the modeled 'true' prevalence of 24.8%. The cure rate of ivermectin was 98.3%. In conclusion, *S. stercoralis* infection is highly prevalent among rural Cambodian schoolchildren. The sensitivity of Koga-Agar technique is higher than Baermann method (88.4% vs. 75.0%). In absence of a "gold standard test", the analyzing of multiple stool samples by different diagnostic methods is required. Ivermectin is highly efficacious against *S. stercoralis* infection and highly cost in Cambodia.

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THE 31 KDA ANTIGEN OF *ANGIOSTRONGYLUS CANTONENSIS* COMPRISES MULTIPLE ANTIGENIC GLYCOPROTEINS

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Human angiostrongyliasis results from accidental infections with intra-arterial nematodes of the genus *Angiostrongylus*. *A. cantonensis* infections result in eosinophilic meningitis and *A. costaricensis* infections cause eosinophilic enteritis. Immunological methods are critical for the diagnosis of both infections since these parasites cannot be isolated from either cerebrospinal fluid or fecal samples. *A. costaricensis* and *A. cantonensis* share common antigenic epitopes which elicit antibodies that recognize proteins present in either species. Detection of antibodies to a 31 kDa *A. cantonensis* protein, present in crude adult worm extracts, is a sensitive and specific method for immunodiagnosis of cerebral angiostrongyliasis. The objective of the present work was to isolate and characterize the 31 kDa protein(s) using soluble protein extracts derived from adult female worms using both single (1DE) and two-dimensional (2DE) gel electrophoresis. Purified proteins were blotted onto nitrocellulose and tested using sera from infected and non-infected controls. The 31 kDa band present in 1DE gels and the 4 spots identified in 2DE gels were excised and analyzed by electrospray ionization mass spectrometry. Four unique immunoreactive proteins with molecular masses close to 31 kDa region were identified based on the highest scores obtained after MASCOT analysis: tropomyosin, the 14-3-3 phosphoserine-binding protein, a nascent polypeptide-associated complex domain, and the putative epsilon subunit of coatomer protein complex isoform 2. Oxidative cleavage of diols using sodium *m*-periodate demonstrated that carbohydrate moieties were essential for the antigenic reaction of all four of the 31 kDa proteins. This data has strong implication for the choice of appropriate vectors to express such recombinant targets for development of diagnostic tests for angiostrongyliasis.

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WHAT CAN FREE-LIVING AND PARASITIC WORMS TELL US ABOUT ANTHELMINTHICS?

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Soil-transmitted helminth (hookworms, *Ascaris* and *Trichuris*) infections are now acknowledged as key contributors to morbidity and poverty worldwide. Although currently there are two approved classes of anthelmintics used to treat human intestinal roundworm parasites, both were initially developed to treat veterinary parasites. We have evaluated *Bacillus thuringiensis* (Bt) crystal (Cry) proteins as novel anthelmintics and considered: 1) how well the effect of a specific anthelmintic on free-living *Caenorhabdits elegans* or the rodent parasite *Heligmosomoides bakeri* might predict efficacy on parasitic roundworms more closely related to those that infect humans; and 2) how the effect of anthelmintics *in vitro* corresponds to that *in vivo*. To address these questions, we have initiated a study of the effects of five different classes of anthelmintics on five different roundworm species, including three in the same genus as human parasites (*Ascaris*, *Trichuris*, *Ancylostoma*) and two not (*Heligmosomoides* and *Caenorhabdits*). We are quantitating the effects of these anthelminthics on viability of all five species *in vitro* and on several *in vivo*. Here we will discuss our work in progress on correlation of anthelmintic effects from roundworm to roundworm, on comparing *in vitro* and *in vivo* results, and on development of Bt Cry protein Cry5B as the novel anthelmintic. We will also discuss the implications of these results for future application of novel anthelmintics for treating human parasitic roundworms.

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CHARACTERIZATION OF ABA-1 EXPRESSION IN EARLY LARVAL STAGES OF *ASCARIS* AND ITS PRESENCE IN HOST FLUIDS IN EARLY AND LATE STAGES OF INFECTION

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Ascaris is a soil transmitted helminth infection that is estimated to affect one sixth of the world's population. Current diagnosis of ascariasis is made using the Kato Katz method of microscopic examination of stool specimens. This is time and labor intensive, varies in sensitivity and specificity depending on the examiner. In addition, diagnosis via stool examination is not possible until one month into the infection when the life cycle is complete. Serological assays have been unreliable because of differences in host responses to Ascaris antigens. The development of an assay that could detect Ascaris antigen in host bodily fluids in the early phase of infection would have wide applicability and utility. Immunoscreening of an *A. suum* infective larval stage 2 cDNA library was performed using sera from infected swine. Nitrocellulose membranes were rinsed, blocked and incubated with primary antibody followed by secondary antibody incubation with anti-pig IgG. BCIP/NBT Sigma Fast was used for staining immune complexes. Only two cDNA clones were strongly recognized by the immune sera. The clones were plaque-purified and their inserts sequenced. These were found to encode different portions of the ABA-1 open reading frame. ABA-1 is an *Ascaris* antigen that has previously been described as a component of the *Ascaris* ES (excretory-secretory) protein which is produced and excreted by all stages of the parasite. Knowledge of the immunodominance of this antigen expressed in early L2 phase of infection will be used to screen timed specimens for the presence of this protein. Recombinant ABA-1 protein obtained from the ABA-1 containing clones will be quantified and used as a control. Mutiple body fluids from infected and control swine will be screened. ABA-1 protein is also expressed by *A. lumbricoides* which infects humans, which makes it an ideal target for use in identifying early infection. The results and their implication for the development of new diagnostic tests of Ascaris infection will also be presented.

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THE THRESHOLD EFFECT: MAGNITUDE AND FREQUENCY OF HOOKWORM LARVAL EXPOSURE DETERMINES THE HOST RESPONSE TO INFECTION

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Hookworm infection affects more than 500 million people worldwide, and represents a major cause of anemia in pregnant women and children. Acquisition of adult worms in the intestine likely results from intermittent exposure to low numbers of infectious larvae via contact with fecally-contaminated soil. Experimental hookworm infection is generally characterized by delivery of a single, relatively large inoculum of third stage larvae (L3) in order to study pathogenesis in a permissive animal model. In an attempt to model the dynamics of naturally-acquired infection more closely, we compared the clinical, immunological and parasitological features of single primary infection (10 L3 vs 100 L3) with twice weekly exposure of hamsters to *Ancylostoma ceylanicum* hookworm larvae. Animals exposed to a multiple high dose (100 L3) larval challenge exhibited similar blood hemoglobin levels, hookworm antigen-specific serum IgG responses, and fecal egg excretion compared to those receiving a single exposure, despite a 20-fold difference in total inoculum. In contrast, animals given repeated low dose (10 L3) exposure had lower blood hemoglobin levels, higher antigen-specific serum IgG responses,

and significantly increased fecal egg excretion compared to their primary challenge counterparts, suggesting continued worm accrual over the course of the 82 day study period. Antigen-specific IgM levels increased throughout the duration of the study in all groups, while IgA antibodies directed at larval proteins peaked in an inoculum-dependent manner 35 days after initial exposure, eventually declining below the detection threshold. These data demonstrate that the frequency and magnitude of hookworm larval exposure influences intensity of infection, pathology, and humoral immune responses to parasite antigens. Furthermore, the data suggest a threshold of exposure below which animals remain susceptible to repeated infection with *A. ceylanicum* hookworms, potentially allowing for more accurate modeling of human infection using a permissive animal host system.

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TRANSCRIPTOME ANALYSIS OF THE ANTERIOR SECRETORY GLANDS OF THE PARASITIC HOOKWORM, *ANCYLOSTOMA CANINUM*

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Hookworms are blood feeding parasitic nematodes that infect almost a billion people around the world. Hookworm disease is characterized by a severe iron and protein-deficiency anemia, malnutrition, and immunosuppression. Currently, there are no FDA approved vaccines for hookworms and deworming chemotherapy does not prevent reinfection. A vaccine is highly desired. The parasitic stage of the hookworm injects potent compounds with immunosuppressive properties, which enable the hookworm to establish chronic infections. The proteinaceous component of the secreted products injected into the host may be the key to a vaccine. However, the identity and origin of many of the secreted proteins are unknown. The purpose of this study was to identify the proteins potentially injected into the host during hookworm parasitism. To identify the proteins expressed in the cephalic and esophageal glands, the head of the parasitic hookworm containing the cephalic and esophageal glands was isolated, and a phage cDNA library was created. In total, 2,350 clones were randomly picked and sequenced using Sanger-based method. The expressed sequence tags (ESTs) were cleaned, clustered, and annotated using dCAS, a semi-automated pipeline for sequence analysis. Functional annotation was added using the BLAST algorithm, and similarity-based searches were performed against various public protein sequence databases. Of the 2,350 clones picked, 1994 were high quality and considered for further analysis. The 1994 ESTs assembled into 673 unique transcripts coding for 511 proteins. The most abundant transcripts expressed in the hookworm head were the excretory/secretory protein 1, predicted to be involved in intracellular trafficking and secretion; the nematode anticoagulant peptide 5, a potent inhibitor of the activated coagulation factor 10 (FXa); platelet inhibitor; the Ancylostoma secreted protein 1, member of the pathogenesis-related protein family; and three unknown proteins with not hits to the NCBI protein database. Of the 673 unique transcripts, 188 had hookworm homologs; the remaining 485 transcripts were novel to the hookworm and their most abundant functional domains were the ShKT toxin domain, with putative potassium channel blocking activity; lectins, putative anticomplement; and many transcripts coding for proteins with unknown function. Future studies will involve functional analysis of abundant transcripts.

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VIRAL LOAD SUPPRESSION IN THE DIRECTLY OBSERVED THERAPY OF HIV SEROPOSITIVE PATIENTS UNDERGOING ANTIRETROVIRAL TREATMENT IN CENTRAL NIGERIA

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Directly observed therapy (DOT) has been identified as a strategy aimed at improving compliance among patients with difficulties adhering to anti retroviral treatment (ART). The rationale for application of DOT in HIV care is based on its successful use in treating non-adherent patients with tuberculosis. The impact of the use of DOT strategy was assessed among 173 HIV sero-positive antiretroviral-naïve patients enrolled for ART at the Jos University Teaching Hospital (JUTH) between March and September 2004. Lamivudine, stavudine and nevirapine combination therapy was administered. Forty six (26.6%) of the patients were placed on daily DOT, 39(22.5%) were on twice weekly DOT, 36 (20.8%) were on once weekly DOT while 52(30.1%) were on self administered therapy. At baseline, the mean weights of the patients were 63.5kg, 59.5kg, 64.3kg and 35.3kg respectively for the various categories. The median CD4+TLC of the various groups were 138cells/µl (range. 10 -356), 138cells/µl (16-334), 100cells/µl, (range 6-340) and 134cells/µl (range 20-362) respectively. The median HIV-1 RNA of the patients were 71,377copies/µl (range 200-3,611,910), 136,302copies/µl (range 200-1,283,250), 186,646copies/µl (range 200-4,472,701) and 149,215copies/µl (range 593-2,675,063) respectively. At the end of 48 weeks, the mean weight of the patients on ART increased to 68.1kg, 68.9kg, 62.1kg and 67.6kg against 63.5kg, 59.5kg, 64.3kg and 62.4kg respectively recorded at baseline in the various categories of treatment. Also the median CD4+ cell counts rose from 138, 138, 100 and 134 cells/ml at baseline in the different categories to 352, 315, 360 and 326 cells/ml respectively at week 48. The viral suppressions (<400copies/ml) among the daily DOT category was 91.9% after 24 weeks and 89.2 at week 48. Among the twice daily DOT group, the suppressions were 74.1% and 85.2% at weeks 24 and 48 respectively. Viral suppressions were 81.3% and 84.4% among the once weekly DOT group after 24 and 48 weeks respectively, while among the self administered therapy group, viral suppressions were 82.1% at week 24 and 79.5% at week 48.

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PRENATAL EXPOSURE TO MALARIA ALTERS EXPRESSION OF SELECTED TRANSCRIPTION FACTORS IN CD4+ MEMORY CBMC THAT INCREASES SUSCEPTIBILITY TO HIV *IN VITRO*

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Recurrent or chronic infections in pregnant women living in malaria endemic areas can activate the fetal immune system *in utero* and may increase risk for mother-to-child transmission of HIV. We have previously shown that unstimulated cord blood mononuclear cells (CBMC) primed to malaria blood stage antigens show increased susceptibility to HIV infection *in vitro* compared to CMBC from non malaria primed offspring. To understand the basis for this increased susceptibility of CBMC to HIV infection, we examined the molecular pathways involved in HIV infection of CBMC subpopulations. We found that effector memory $CD4^+$ T cells were the exclusive initial targets of HIV infection, with rapid viral spread to the central memory compartment. Increased expression of CD25 and

HLA-DR was observed on both central and effector memory cells of HIV susceptible vs. not susceptible CBMC indicating ex vivo activation is important in viral susceptibility. This increased susceptibility was not associated with increased viral entry of target cells since detection of minus strand strong-stop DNA twenty-four hours post virus exposure was similar in all samples tested. By contrast *gag/pol* RNA was only detected in HIV susceptible CBMC samples, suggesting that integration or gene transcription of integrated DNA provirus regulates susceptibility. To examine these possibilities we performed a targeted gene expression analysis of the total memory population by PCR array, which reproducibly showed greater expression of *IFNg*, *NFATc1, IRF1, FOS*, and *PPIA* and decreased expression *YY1* and *TFCP2* in HIV susceptible vs. not susceptible CBMC. This suggests that in malaria primed CBMC, activation of host genes that regulate integrated proviral gene transcription increase susceptibility to HIV infection. This system provides a valuable model to understand critical pathways that affect T cell susceptibility for HIV replication *in vivo* and has broader implications that efforts to reduce maternal co-infections during pregnancy may help reduce risk for vertical transmission of HIV.

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PRELIMINARY STUDIES ON HIV-1 ASSOCIATED IMMUNE RECONSTITUTION INFLAMMATORY SYNDROME IN CHINESE HIV INFECTED INDIVIDUALS

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Immune Reconstitution Inflammatory Syndrome (IRIS) is the paradoxical inflammatory syndrome developed soon after antiretroviral therapy initiation. Although many recent studies have reported variable incidence of IRIS, in china however, limited data on clinical and mechanism of IRIS are available. The aim of the present study was to investigate the the immunological and biological factors involved in occurrence in AIDS patients after HAART initiation. 238 AIDS patients who received initial HAART were followed for IRIS over 24 weeks. Clinical manifestations, T-regs, Th1/Th2 cytokines and IL-7 were monitored at base line, onset of IRIS, week 4, 12, 24. RESULTS: IRIS occurred in 47 patients (19.7%) within 28 (9-36) days after HAART initiation. The first case appears only 5 days after HAART initiation and the last case, 150 days later. Systemic OI (OI-IRIS) accounted for (19.7%; 47/238) of IRIS cases, predominantly of Tuberculosis (29 cases), Herpes simplex (8 cases), Herpes zoster (5 cases), Cytomegalovirus (2 cases), Cryptococcal Encephalitis (1 cases). CD4+/CD8+ naive and memory T cells exhibited no significant differences between both groups however, CD4+CD25+Foxp3+ regulatory T cells decreased in IRIS group compared to non-IRIS group. IL-2 and IFN-γ were significantly higher in IRIS group whereas IL-4 and IL-10 were significantly lower in IRIS group. IL-7 decreased gradually during HAART, but was higher in IRIS group during all the follow-up. In conclusion, according to our results, antecedents of opportunistic infections, baseline low CD4 cell count associated to an imbalance of Th1/Th2 cytokine with increased IL-7 may be determinant for IRIS occurrence.

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A UNIQUE INFLAMMATORY PATTERN IN THE BRAINS OF HIV-1 SEROPOSITIVE CHILDREN DYING FROM CEREBRAL MALARIA

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Malaria deaths occur primarily in children in sub-Saharan Africa and are due to severe malaria including cerebral malaria (CM). In Malawi, where the entire population is at risk for malaria, HIV-1 prevalence is 12%. High rates of malaria/HIV coinfection are likely but the effects of HIV on CM pathogenesis and outcome are virtually unknown. Comparing brain pathology of HIV-infected (HIV+) and -uninfected (HIV-) individuals with clinically-defined CM could help identify differences in pathogenesis. The Blantyre Malaria Project (BMP) has found 3 patterns of pathology in children meeting WHO criteria for CM: intravascular parasites alone (CM1), intravascular parasites and parenchymal ring hemorrhages (CM2) and no pathology suggestive of CM (CM3, or faux CM). In this cohort the HIV+ rate is higher among autopsies than in the total cohort (20% vs. 13%) and 57% of autopsies with the CM1 pattern are HIV+ compared to 18% with CM2. Because of the association of HIV with the CM1 pattern we performed immunohistochemistry on brain tissue from a subset of the BMP cohort with clinically-defined CM. These included 10 subjects with the CM1 pattern, 10 with CM2 and 10 with faux CM. Five from each group were HIV+ by antibody-based test. We labeled for HIV-1 p24 and ionized calcium binding adapter molecule 1 (Iba1), a marker expressed in activated microglia and monocytes. No HIV-1 p24 was seen. We found a unique pattern of Iba1+ intravascular monocytes more frequently in HIV+ (8/10) than in HIV- (4/10) subjects. These cells frequently contain hemozoin, appear to completely occupy small vessels and adhere to the walls of larger vessels. In the CM1 group, 5/5 HIV+ and 3/5 HIV- subjects had intravascular Iba1+ cells. In the CM2 group, intravascular Iba1+ cells were seen in 3/5 HIV+ and 1/5 HIV- subjects. There were no intravascular Iba1+ cells seen in the CM3 group regardless of HIV status. We found a unique inflammatory pattern characterized by intravascular monocytes, more frequently seen in HIV+ children dying from CM. Efforts to quantify these cells and further characterize them by other surface markers are ongoing.

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BRAIN ABSCESS DUE TO *SALMONELLA TYPHIMURIUM* AND *MYCOBACTERIUM TUBERCULOSIS* IN A PATIENT WITH AIDS

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The incidence of nontyphoid *Salmonella* infections is common in AIDS patients. However, *Salmonella* infections in the Central Nervous System (CNS) are rare, even amongst HIV positive patients. Tuberculosis is another infection that has reemerged with the advent of AIDS. Ten to 20% of cases of AIDS-related extrapulmonary tuberculosis involve the CNS, but brain abscess due to *Mycobacterium tuberculosis* is rare, with few cases described in literature. We describe a case of a 38 years old man who was diagnosed with HIV in 1999, when he presented with *Toxoplasma* encephalitis. He had an irregular use of the antiretrovirals drugs, and in 2005, he was treated for a tuberculous lymphadenitis, and later a diarrhea and meningitis, both caused by *Salmonella*. In September, four months after the treatment of the *Salmonella* infection, the patient presented with seizures. He was hospitalized and a brain CT scan demonstrated two lesions with contrast ring-enhancement. An empirical treatment for *Toxoplasma* encephalitis, using sulfadiazine, pyrimethamine, and folinic acid, was introduced, but 12 days later the patient had no clinical

improvement and developed mental confusion. The new brain CT scan demonstrated an increase in the size of the lesions. He underwent a brain biopsy draining 15 ml of purulent material. The culture of this secretion was positive *for Salmonella typhimurium* and *Mycobacterium tuberculosis*. The patient was treated for 60 days with ceftriaxone, and specific drugs for tuberculosis were introduced later (rifampicin, isoniazid, pyrazinamide and ethambutol). One year after the diagnosis of brain abscess, the patient still showed residual lesions on CT scan but an important clinical improvement. *Mycobacterium tuberculosis* and *Salmonella* are rare even as an individual etiologic agent of brain abscess and there is no other case in the literature of both microorganisms in the same CNS lesion. In this case we suggested the *Salmonella* treatment maintenance with ciprofloxacin, until immunological improvement was achieved with the use of antiretrovirals.

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MULTIPLE MYELOMA IN A PATIENT WITH AIDS

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Several haematological neoplastic diseases and solid tumors have been associated with HIV infection, such as Kaposi's sarcoma, non-Hodgkin lymphoma (NHL) and cervical cancer. The association of Multiple Myeloma (MM), a malignancy of post-germinal centre B cells, with AIDS has been controversial and there have been found only a few cases described to date. We reported here a 28-year-old HIV-infected woman who was regularly receiving antiretrovirals drugs (tenofovir, lamivudine, efavirenz, atazanavir and ritonavir) and presented a CD4+ lymphocyte count of 155 cells/mm^3 and HIV viral load < 50 copies/mm^3. She was referred in October 2007 for investigation of reduced muscle strength of the right hemibody, and infraclavicular and scalp nodular lesions. CT brain scan showed multiple lytic skull lesions and a soft part density nodule located in the left high convexity parietal, associated with meningeal enhancement. The thoracic radiography showed multiple costal aches lytic lesions. Bone marrow biopsy was normal. Serum protein electrophoresis showed IgG 628 mg/dl (770-1510 mg/dl), IgA 2440 mg/dl (134-297 mg/dl) and IgM 50,7 (67-208 mg/dl) and biopsy of infraclavicular and scalp lesions showed plasmablastic plasmocytoma with clone restriction of lambda light chain. A diagnosis of MM was made and the patient started thalidomide and dexamethasone cycles. After 2 months, the patient presented significant reduction of the lesions and a decrease in IgA levels to 135 mg/dl. In the future, as HIV patients have access to potent antiretrovirals drugs and undergo immune reconstitution, there may be more cases of MM rather than NHL. It would be pertinent to consider MM as part of the differential diagnosis in HIV-associated clinical manifestations and to exclude HIV infection in young patients presenting with myeloma.

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USING *LISTERIA* VECTORS TO OVERCOME HELMINTH INFECTION: GENERATING TH1 VACCINE RESPONSES IN TH2 BIASED, IMMUNE SUPPRESSED HOSTS

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Malaria, TB and HIV remain tremendous disease burdens in much of the world's population and functional vaccines are desperately needed. Although sub-Saharan populations are those that will benefit most from these vaccines, they are also coincident with areas endemic for helminth infection. Infection with one or more species of parasitic helminths may suppress the immune system and has been shown, by our lab and others, to suppress vaccine-specific responses. One goal of our research is to find vaccines that will drive significant vaccine-specific immune responses in helminth infected recipients without the need to eliminate helminth infection prior to vaccination. In the current study, we demonstrate that administration of a *Listeria* vector HIV-1 gag vaccine to mice chronically infected with the helminth parasite *Schistosoma mansoni,* drives significant immune responses to HIV-1 gag CTL and helper epitopes. This observation suggests that *Listeria* vector vaccines are capable of driving vaccine-specific responses in helminth-infected populations. Kinetic studies show the antigen-specific responses are durable and induce CD8+ central memory. Based on these observations, we believe *Listeria* vectors should be considered in the development of new generation HIV-1, malaria or TB vaccines to be administered to populations in sub-Saharan Africa where helminth infection is endemic. Studies are underway to determine if other vectors are also capable of overcoming helminth-induced immune suppression.

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HELMINTH ANTIGENS AS ADJUVANTS FOR HIV-1 VACCINES

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Helminth parasites bias the host immune system towards Th2-type and often induce immune suppression, which has been shown, by our lab and others, to inhibit Th1 vaccine-specific responses. Previous studies have shown the complex mixture of molecules that comprise saline soluble egg antigens (SEA) from *Schistosoma mansoni* function to induce Th2-biasing in naïve individuals. SEA has been used experimentally as Th2-type adjuvant for vaccine antigens. In preliminary studies, we asked if co-administration of SEA with a *Listeria* vector HIV-1 gag vaccine in mice, would suppress host cytotoxic T lymphocyte (CTL) and T helper responses to the HIV-1 gag epitopes. Although co-administration of SEA did bias the host immune system towards Th2-type, unexpectedly, co-administration of SEA with the *Listeria* vector HIV-1 gag vaccine significantly increased the frequency of IFN-γ producing gag-specific T helper and CTL responses over that seen in mice that received only the vaccine. This result suggests that there are components in SEA that are potent inducers of Th1-type responses, which, if identified, could be utilized as adjuvants to promote Th1-type vaccine-specific immune responses for HIV-1 and other vaccines. We are continuing to examine the adjuvant properties of SEA and determine which class(es) of molecules in SEA promotes Th1-type immune responses.

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LOOKING FOR SEX WORKERS IN THE GOLD MINING AREAS OF SURINAME: AN ENUMERATION STUDY

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HIV transmission in Suriname mainly takes place through unprotected sex. Higher HIV prevalence in groups with multiple partners having unprotected sex e.g. Sex Workers (SWs), favor the spread of HIV because of the bridge to the rest of the population. The National Strategic Plan identifies Sex workers as a target group for HIV intervention activities. For advocacy, good planning of intervention activities and the evaluation of HIV program, a valid and reliable estimate of target population is needed. In the last decade small-scale gold mining activities in the interior of Suriname has increased, assumingly leading to a higher influx of SWs to these areas. As part of a size estimation study of Sex workers in Suriname, a rapid ethnographic mapping guided by key informants and gate keepers was done in selected gold mining areas, with a prospective high concentration of SWs. Two localities outside the gold mining areas, with known presence of SWs offering services to gold miners, were also included. Every location was visited by 2 interviewers together with a "resource person" (someone familiar with the population at the site and trusted by them). During a 4 week period, questionnaires were handed out to 192 (189 and 3) consenting SW in the age range of

15 to 49 year. 60% was between 20 - 29 years. Of the SWs surveyed, 58.3% was Brazilian, 28.1% Surinamese, 12% Dominican and 1.6% Columbian. Junior high or lower education level was found in 77.6% of interviewees. 50.4% started sex work at age ≤ 19 year. Looking at "safe sex practices", 78.6% always use a condom and 90% had ever taken an HIV test of which 58.3% in the last year. Covering all areas with high density of people, 192 sex workers were found in the gold mining areas of Suriname. Not included here are the women who primarily do other work (such as cooks, shop keepers etc.), but who according to anecdotal data, also exchange sex for money or goods. From a HIV prevention perspective this is also an important group and additional research is certainly needed.

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TREND IN THE PREVALENCE OF HIV/AIDS IN THE STATE OF MISSISSIPPI: A FIVE YEAR REVIEW

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Acquired immune deficiency syndrome (AIDS) remains a disease of grave concern all over the world caused by the human immunodeficiency virus (HIV). It is one of the dreaded sexually transmitted diseases (STDS) but which can also be spread by contact with infected blood, from mother to child during pregnancy, childbirth, or breast feeding. There is no current cure for it but there are antiviral drugs that can ameliorate its severity. The symptoms vary depending on the phase of infection. Mississippi with a population of 2.9 million is one of the states where HIV/AIDS is most prevalent. The Purpose of this study is to assess the trend in the prevalence of HIV/AIDS in the last five years (2006 - 2010). The study is based on the statistical analysis of the prevalence reports in literature and the Mississippi State Department of Health. The literature review shows that the prevalence of HIV/AIDS in the State of Mississippi in the last five years appears to have plateaued. There is no significant difference on year to year basis from 2006 to 2010 ($P > 0.05$). HIV infection by sex showed a preponderance of males infected as against females (68.4% and 31.6% respectively) for the five years under review. It also shows that reported cases of individuals living with HIV/AIDS by year did not show significant differences ($P > 0.05$). The cumulative cases of HIV/AIDS in the State of Mississippi from 1983 to 2009 are 12,989. Of this number 3,263 (26.1%) is white, 9393 (72.3%) is African American, and 197 (1.5%) is Hispanic. These results are very revealing. The trend shows that the prevalence is high in African Americans. With the exception of a slight decrease in 2010, it appears to be increasing. It is much less in whites and appears to be decreasing. More efforts need to be made to control HIV/AIDS among African Americans.

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COST OF ISONIAZID PREVENTIVE THERAPY WITH AND WITHOUT TUBERCULIN SKIN TESTING AMONG HIV CLINIC PATIENTS IN RIO DE JANEIRO, BRAZIL

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The WHO recommends isoniazid preventive therapy (IPT) for all individuals with HIV once active tuberculosis (TB) is excluded. Brazil is a high TB-burden country whose national guidelines recommend use of tuberculin skin testing (TST) to identify individuals eligible for therapy, but IPT use has been limited. In 2005, the Consortium to Respond Effectively to the AIDS/TB Epidemic began a study (THRio) of IPT in HIV clinics in Rio de Janeiro in order to determine if routine screening for and treatment of latent TB in HIV patients reduces TB incidence in the clinic population. While use of TST and IPT improved under THRio, patients continue to experience significant delay to TST and IPT initiation, which eliminating the TST may reduce. Prevalence and incidence data from THRio and published literature were used to estimate TB incidence, TST, IPT, and HAART coverage in order to determine the effectiveness and incremental program cost of increasing TST and IPT use as well as providing IPT without prior TST. Modeling the expected annual incident TB cases from a hypothetical cohort of 10,000 HIV-positive clinic patients demonstrates that the THRio intervention results in a 6% annual reduction in TB cases with 22 cases averted from baseline, while providing IPT without TST results in a 50% annual reduction, with 176 cases averted. Using costs from published literature and online data, and considering the potential number of cases averted, a cost analysis demonstrates that for a program evaluating 10,000 patients per year, increasing TST coverage to 60% results in an increase in cost of US$38,757.52 in the first year. Conversely, providing IPT without TST to all patients, assuming 75% coverage, decreases annual cost by US$146,127.83 in the first year. Over a five-year period, adjusting for inflation, increasing TST coverage to 60% increases cost by nearly US$200,000, while providing IPT without TST to all patients, assuming 75% coverage, decreases cost by over US$600,000. This analysis suggests that providing IPT to HIV clinic patients without prior TST is the most beneficial strategy regarding number of cases averted and program cost in a high TB burden region.

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PLACENTAL *PLASMODIUM FALCIPARUM* MALARIA INFECTION: FIELD ACCCURACY OF HRP-2 RAPID DIAGNOSTIC TESTS IN AN ENDEMIC SETTING

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It is widely recognized that malaria has a negative effect on the outcome of pregnancy. Pregnant women with little or no pre-existing immunity are at high risk of cerebral malaria, hypoglycemia, pulmonary edema, and severe hemolytic anemia, and fetal and perinatal loss can be as high as 60-70%. However, peripheral blood smear microscopy is not always able to detect malaria parasites due to their sequestration in the placenta. Use of malaria rapid diagnostic tests (RDTs) detecting Histidine Rich Protein-2 antigen (HRP-2) in peripheral blood are a potential alternative. In an endemic setting in Uganda, we compared the accuracy of HRP-2 RDTs to microscopy and placental histopathology in pregnancy. Discordant results samples were spot checked using PCR techniques. Among 434 febrile women tested, 38% had malaria. RDTs had a sensitivity of 96.8% (95% CI 92-98.8), specificity of 73.5% (95% CI 67.8-78.6), a positive predictive value (PPV) of 68.0% (95% CI 61.4-73.9), and negative predictive value (NPV) of 97.5% (95% CI 94.0-99.0) in detecting peripheral *Plasmodium falciparum* malaria during pregnancy. Mosquito net use (OR 2.1) and increasing parity (OR 2.7) were associated with lower risk for malaria. At delivery, RDTs had a 80.9% sensitivity (95% CI 57.4-93.7) and a 87.5% specificity (95%CI 80.9-92.1), PPV of 47.2 (95% CI 30.7-64.2) and NPV of 97.1(95% CI 92.2-99.1) in detecting placental *P. falciparum* infections. At delivery, 25% of peripheral infections were detected by microscopy without concurrent placental infection. Compared to placental histopathology, the combination of RDTs and microscopy improved the sensitivity to 90.5% (95%CI 68.2-98.3) for detecting placental malaria infection and the specificity to 98.4% (95%CI 93.9-99.7). Presence of malaria in pregnancy and active placental malaria infection were 38% and 12% respectively. Use of HRP-2 RDTs to detect malaria in pregnancy was accurate when performed by midwives. A combination of RDTs and microscopy provided the best means of detection placental malaria. With a high sensitivity, RDTs could be an useful tool for assessing Malaria in

pregnancy, further research, including (cost-)effectiveness studies will be needed to assess the potential role off RDTs in malaria in pregnancy control.

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PARASITEMIA INDEX IN PATIENTS INFECTED WITH *PLASMODIUM FALCIPARUM* MALARIA

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Severe *Plasmodium falciparum* malaria known as a medical emergency, the treatment requires institution of intensive care as clinical manifestation of severe *P. falciparum* malaria patients is highly diverse and complex. According to the World Health Organization (WHO) criteria, many factors are utilized for definition of severe malaria. Parasitemia density is an important manifestation for severe malaria determination; however, at present there is no uniform agreement for hyperparasitemia definition to define severe malaria. This study was undertaken to illustrate the clinical manifestations as well as to establish the cutoff point of parasitemia density in *P. falciparum* malaria patients for definition of severe malaria. The presenting clinical manifestations of *P. falciparum* malaria patients were analyzed in relation with parasitemia density. 389 malaria patients, admitted at The Bangkok Hospital for Tropical Diseases, were studied. According to WHO's criteria 2006, 200 cases defined as uncomplicated malaria and 189 cases were severe malaria. Regarding to the statistical methods, it was observed that 1% parasitemia gave the most optimal sensitivity and specificity of 79.3 and 73.5, respectively with accuracy of 76.3%. In addition, we found that 1 % parasitemia revealed a statistically significant association with disease severity, low platelet counts, increasing of blood urea nitrogen and creatinine, increased of serum transaminases, jaundice, pulmonary edema, metabolic acidosis, prostration and schizontemia. In conclusion, presenting syndromes of severe *falciparum* malaria depend on many factors. For hyperparasitemia definition, 1% of parasitemia infected red blood cell could be considered as a cutoff point for severity definition, particularly in low transmission area.

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HOW MUCH REDUCTION IN THE OVER-DIAGNOSIS OF MALARIA CAN BE EXPECTED WITH THE USE OF TESTS IN RURAL GHANA?

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To reduce the over-diagnosis of malaria, WHO now recommends that diagnosis be confirmed by tests in all transmission settings. We conducted a cross-sectional study in a district hospital in rural Ghana (from January 2009 to February 2010) to assess the extent of over-diagnosis of malaria and how much reduction could potentially be achieved with the use of rapid diagnostic test (RDT) or microscopy. Under-five children presenting with a history of fever were managed presumptively while samples were taken for malaria RDT and smear microscopy. A total of 936 children were enrolled: 775 in the wet season and 161 in the dry season. Overall 689 (73.6%) were presumptively diagnosed with malaria. Had diagnosis been based on rapid diagnostic test or microscopy, 618 (66.0%) and 404 (43.2%) cases respectively would have been diagnosed with malaria. Using RDT in the wet and dry seasons, reduction in malaria diagnosis would have been 4.1% and 24.2% (diff 20.1%, CI 13.3% - 26.9%, p<0.001) respectively. With microscopy, the reduction would have been 30.5% and 29.8% (0.7%, CI -7.1% - 8.5%, p=0.86) respectively. Using microscopy as standard, the sensitivity and specificity of the RDT used were 97.7% and 58.1% respectively. The anticipated reduction in malaria over-diagnosis may be limited by the low specificity of RDTs and their cost-effectiveness is likely to be season-dependent.

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PERSISTENCE OF *PLASMODIUM OVALE CURTISI, P. OVALE WALLIKERI* AND *P, MALARIAE* IN ASYMPTOMATIC GHANAIAN SCHOOL CHILDREN TREATED WITH DHA-PIPERAQUINE

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Microscopy remains the gold standard for the diagnosis of malaria in the field despite its limitations. However, there remains an under estimation of the true malaria burden especially of less prevalent and less documented species such as *Plasmodium ovale* sp. and *P. malariae*, both of which frequently exist as low density infections. We used standard species-specific nested polymerase chain reaction, targeting the small subunit ribosomal RNA gene, to investigate the presence of malaria parasites in a total of 274 filter paper blood spots from asymptomatic school children in Pokukrom in the Ahafo Ano South district, Ashanti region, in the Southern zone of Ghana. One hundred and forty-five pupils who were microscopically positive for *P. falciparum* asexual parasitaemia were subsequently treated with dihydroartemisinin piperaquine and followed up for 28 days. Of the 274 pre-enrolment samples analyzed, 210 (77%) were positive for *P. falciparum*. Many of the infections were shown by PCR to be comprised of multiple species with 44 (16%) also harbouring *P. ovale* sp., and 77 (28%) harbouring *P. malariae*. There was no evidence of *P. vivax* in our study participants. *P. ovale* positive samples were further classified into *P.o.curtisi* and *P.o.wallikeri* by nested PCR at two different loci (Plasmodium ovale tryptophan-rich antigen (Potra) and Plasmodium ovale glyceraldehydes-3-phosphatase (Pog3p) followed by sequence analysis. In a small number of cases, recurrent parasites were detected by species-specific PCR 28 days after treatment. All three species were represented, unexpectedly, with cases of ovale (4 of 44) and malariae (3 of 77) recurrence, confirmed by PCR. This is the first report of recurrence of these species within 28 days of ACT treatment. There is an urgent need to improve diagnosis of these overlooked non-*falciparum* malaria parasites and to determine their *in vivo* sensitivity to currently used antimalarial drugs. *P. ovale* sp. and *P. malariae* are common throughout sub-Saharan Africa, and thus are important targets for malaria control and elimination.

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SUPERVISORY VISITS IN BENIN SHOW IMPROVEMENTS IN MALARIA MICROSCOPY

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Researchers have suggested that, "supervision and audit with feedback is generally effective," in achieving and maintaining high-quality performance of health workers in low-resource settings (published reports). In FY2010 IMaD conducted regular, quarterly visits to 60 health facilities in Benin. Due to the staggered approach taken by IMaD for scaling up the number of facilities, only 36 of the 60 health facilities were visited a total of four times by the end of FY2010. During each round of supervisory visits IMaD supervisors, who were previously trained in health facility evaluations and evaluated for malaria microscopy competency, collected information on the current state of health facility practices pertaining to malaria diagnostics and treatment. During these visits, supervisors also provided on-the-job training for individual staff members where deficiencies in their performance in conducting routine

diagnostic procedures (e.g., slide preparation, staining and reading), general laboratory practices (e.g., record keeping, inventory, QA/QC) or treatment of malaria (e.g., discussion of proper treatment) were detected. Supervisors provided comments and feedback to health facility staff during each visit, and suggested methods of improving specific practices when necessary. Maintaining continuity with respect to the cadre of supervisors routinely conducting the visits ensures accountability among the health facility staff to improve performance while concurrently fostering a reliable system of support. Over the course of four visits the percentage of health facilities performing microscopy in full consistency with national guidelines increased from 58.2% to 100% by the fourth of the FY2010 visits. Another figure suggestive of the positive impact is the decrease of antimalarial prescriptions to individuals with negative malaria results, which is, alternatively, a measure of prescriber adherence to malaria laboratory tests. The percentage of health facilities that prescribed antimalarials to negative patients fell from 73.1% to 40% by the end of the FY2010 supervisory visits.

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COMPETENCY AND PROFICIENCY ASSESSMENT CAN IMPROVE PARASITE DETECTION AND SPECIES IDENTIFICATION

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Malaria is the leading course of morbidity and mortality in sub-Saharan Africa responsible for 90% of annual global burden. Accurate diagnosis of malaria is important to ensure correct case management. Studies have shown that microscopy in field conditions has a sensitivity of 68.6% and specificity of 61.5%. AMREF and WHO AFRO introduced a competency assessment course for malaria microscopists based on the model approved by the WHO to assess microscopists. Methodology: A five day training course comprising theoretical lectures and laboratory practical sessions were developed based on the WHO recommendations. Well characterized slides sets were used. Pre and post course practical evaluations consisted of 16 and 55 slides respectively. Twenty slides were negative, ten contained *Plasmodium falciparum* with parasite density range 80-200 parasites/mL, and ten slides had *P. malariae*, *P. vivax* and mixed parasite species. Fifteen slides containing *P. falciparum* were used to assess parasite quantification. Results: Eighty five microscopists have participated from 15 countries. Overall, species identification marginally improved from 51.3% - 71.7% (mean 20.4%, 95% CI 04-41; p= 050). Sensitivity significantly improved from 57% - 91% (mean 34%, 95% CI 17-50%; p=0.003), while specificity improved from 62.2- 90.7% (mean 28.5% 95% CI 10-41%; p=010). Parasite quantification improved from 28.3 - 41.7% (mean 13% 95% CI 8-18%; p<0.00.1). Conclusion: The data shows that participation in proficiency testing programmes can improve performance in malaria parasite detection and species identification. There is a need to translate training materials into French and Portuguese to expand the training in Africa.

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ADHERENCE OF HEALTH CARE WORKERS TO MALARIA RAPID DIAGNOSTIC TESTS IN FEVER PATIENTS ATTENDING PRIMARY HEALTH CARE FACILITIES IN ZANZIBAR

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Zanzibar has recently undergone a rapid transition from high to low malaria transmission. In the new epidemiological context it is critical to target malaria treatment, i.e. artemisinin-based combination therapy (ACT), to patients with confirmed malaria infection. To improve fever case management Zanzibar has introduced malaria rapid diagnostic tests (RDT) in all public health care facilities. This study aimed to evaluate health care workers' adherence to RDT in Zanzibar. The study was conducted in 12 public health facilities, 6 each in North A and Micheweni districts. Prior to the study start all health workers were trained in the recently adapted integrated management of childhood illness (IMCI) guidelines as well as standard malaria treatment guidelines. We enrolled 3893 patients, 1824 5 years of age with fever or history of fever in the preceding 24 hours between May and August 2010. All patients were tested with RDT. Overall 122 (3.1%) patients were RDT positive, of whom 38 were <5 and 86 >5 years of age. Among the 3771 RDT negative patients only 2 (both >5 years) were prescribed ACT. Some 121 of 122 RDT positive patients received treatment with antimalarial drugs, 116 with ACT, 4 with quinine and the remaining patient with ACT and quinine. In conclusion, adherence to RDTs results among health care workers in Zanzibar was excellent in the new epidemiological context with low malaria transmission.

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RAPID DIAGNOSTIC TESTS IN THE CAMBODIAN PRIVATE SECTOR: HOW (WELL) ARE THEY BEING USED IN PRACTICE?

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Access to good quality Artemisinin-based Combination Therapy (ACTs) has recently been given a boost with the launch of the Affordable Medicines Facility malaria (AMFM) pilot, which supports a manufacturer level subsidy for the provision of ACTs through public, non-governmental organisation and private sector channels. In the mean time, there has been a dramatic decrease in malaria in many malaria-endemic countries, increasing the need for better targeting of the drugs. The increasing availability of cheap, reliable malaria Rapid Diagnostic Tests (RDTs) means that serious consideration is now being given to the use RDTs outside of public health facilities, including the private sector. However programmatic experience of RDT in this sector is limited. In 2004 Cambodia became the first country to implement a nationwide programme of subsidised and socially market malaria Rapid Diagnostic Tests (RDTs). A combination *Plasmodium falciparum*/non-*falciparum* test is currently sold from Population Sciences International (PSI) to wholesalers and retailers for $0.50 for a box of ten, allowing for a substantial profit. However, little is known about how the RDTs have actually been used in practice. In late 2010 we carried out a drug outlet survey, RDT user assessment, RDT quality assessment and mystery client study in order to document current practice and quality. Over half of the 217 providers interviewed sold RDTs, the vast majority being the socially marketed brand. They were generally stored in adequate conditions and quality of the tested RDTs was good. Providers appeared to be aware of the need for blood testing and reported few problems although observation of their use suggested there was some

areas in which improvements could be made including time-keeping and safe disposal. We discuss the implications of the findings for future implementation in Cambodia and beyond.

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COST-EFFECTIVENESS ANALYSIS OF INTRODUCING RAPID DIAGNOSTIC TESTS (RDTS) FOR MALARIA DIAGNOSIS IN PUBLIC HEALTH CENTERS WHERE MICROSCOPY IS AVAILABLE AND PERIPHERAL CLINICS WHERE ONLY CLINICAL DIAGNOSIS IS AVAILABLE: THE CASE OF GHANA

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Overdiagnosis of malaria is a problem in most parts of Africa. Current evidence in Ghana suggests that overprescription of antimalarials occurs in public health facilities both where microscopy is available and where diagnosis is done presumptively due to lack of parasitological testing facilities. Introducing rapid diagnostic tests (RDTs) for malaria in public health facilities in Ghana may potentially improve diagnosis and may therefore also be a cost-effective intervention. This study was designed to assess the cost-effectiveness of introduction of RDTs in three public health facilities in Dangme West district of Ghana. Suspected malaria patients attending a health facility with a functioning microscope were randomly assigned to diagnosis by either an RDT or microscopy and subsequent treatment by health centre staff whereas suspected malaria patients visiting two other health centres without microscopy were randomly assigned to diagnosis by an RDT or presumptive diagnosis based on clinical signs. Costs of offering diagnostic services and outpatient services were collected through visits to the health facilities. An exit survey among patients with suspected malaria was used to capture and subsequently cost the drugs prescribed irrespective of final diagnosis. Patients were followed up two weeks later in their homes to inquire about any additional health care seeking since the first visit and the associated household costs. The measure of effect was the number of correctly treated patients by diagnostic arm as determined by a double read blood slide. Among the suspected malaria patients visiting the health facility where a microscope was available, it was found that the proportion of correctly treated patients was similar between the RDT and the microscopy arms and that the costs per correctly treated patient were at a similar level. In the two health centres with no microscope, the proportion of correctly treated patients was higher and the costs lower in the RDT arm as compared to the clinical diagnosis arm.

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THE BENEFITS AND PITFALLS OF EX VIVO AND *IN VITRO* SUSCEPTIBILITY TESTING OF *PLASMODIUM FALCIPARUM* CLINICAL ISOLATES

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Drug susceptibility testing of *Plasmodium falciparum* isolates has not been established to the standard currently used in bacteriology, virology, or mycology. No breakpoints have been determined for the interpretation of IC_{50} susceptibility testing results. Different testing modalities exist and are challenged by time-consuming and laborious culture adaptation procedures. One possible solution has been ex vivo testing of patient blood without culture adaptation. We conducted a three year long effort to establish antimalarial drug susceptibility testing of clinical isolates in Toronto, Canada in returning travelers. Testing was conducted using both the SYBR green *in vitro* and HRP ex vivo methods. IC_{50} data for chloroquine, mefloquine, atovaquone, and artemisinin derivatives were obtained for clinical isolates and compared to reference strains 3D7 and W2. Isolates were also sequenced for single nucleotide polymorphisms previously linked to antimalarial resistance. Our results demonstrate that in the main IC_{50} data from ex vivo and *in vitro* methods do correlate well. However, in certain instances polyclonal infections can confound testing results where selection for a fit parasite clone affects the outcome of both IC_{50} and SNP genotyping. The concomitant testing of control strains 3D7 and W2 enable IC_{50} data to be presented as a ratio. This is important as the IC_{50} result can vary by run and method. Exchange of strain panels between reference laboratories is also essential to the maintenance of quality assurance. We conclude that a set panel of strains and isolates be established for quality assurance purposes and that efforts be augmented to correlate IC_{50} data with clinical outcomes in order to establish clinical breakpoints.

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KINETICS OF PARASITE CLEARANCE TIME BY QUANTITATIVE REAL-TIME PCR AND MICROSCOPY IN SUBJECTS WITH UNCOMPLICATED *FALCIPARUM* MALARIA

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Malaria microscopy, performed on Giemsa stained thick and thin smears, has long been the accepted gold standard for detection of parasites in both the clinic and the field. However, we hypothesized that species-specific real time (RT) PCR would enhance existing methods of parasite detection to confirm low-level parasitemia in settings such as clinical trials where they could be of clinical relevance in predicting outcomes. In a randomized, open label clinical trial conducted in western Cambodia, we assessed a species-specific 18s rRNA genomic DNA RT-PCR assay developed in-house, and compared results with expert microscopy. One hundred forty three subjects with uncomplicated *P. falciparum* malaria were randomized to receive 1 of 3 artesunate monotherapy regimens. Blood for microscopy and RT-PCR was collected pre-treatment, at 2, 4, 6, 8, 12, 18 and 24 hours after the first dose, then every 6 hours until 2 successive slides were negative by microscopy, then daily until discharge on Day 6, and then weekly until Day 42. Geometric mean (95% CI) parasite clearance times were 69.7 (64.8-74.9), 88.8 (79.1-99.6) and 150.5 (130.8-173.2) hours for microscopy, by genus specific RT-PCR, and by species-specific RT-PCR respectively (p=0.0001). The percentage of subjects remaining parasitemic at 72 hours after treatment began was 51, 73 and 89% for microscopy, genus specific RT-PCR and by species-specific RT-PCR, respectively. In most subjects who subsequently failed treatment, both qRT-PCR and microscopy became negative before the day of failure. However, among the failures, several were positive for malaria earlier by qRT-PCR than by microscopy. These data suggest that determination of parasitemia at 72 hours by qRT-PCR is a more sensitive indicator of parasite clearance than microscopy, and that, in spite of the increased sensitivity recrudescent parasites still fall below the limit of detection for this assay. However, this argues for a role of qRT-PCR in clinical trials of antimalarial therapy, in addition to more traditional clinical endpoints.

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IN VITRO METABOLISM-LINKED HEMOTOXICITY ASSAY: VALIDATION AND APPLICATION OF THE ASSAY TO SCREEN NEW ANALOGS AND UNDERSTAND THE MECHANISM OF HEMOLYTIC TOXICITY OF 8-AMINOQUINOLINE ANTIPARASITICS

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Metabolites generated through cytochrome P_{450}-dependent metabolic reactions are responsible for hemolytic effects of primaquine (PQ) and other 8-aminoquinolines (8-AQs). The hemotoxic response of the metabolites generated *in situ* could be measured by estimation of accumulation of methemoglobin (mtHb), kinetic measurement of increase in oxidative stress, and depletion of reduced glutathione (GSH) in a microsomal metabolism-linked hemotoxicity assay (Ganesan *et al*, Toxicol Appl Pharmacol. 2009; 241:14-22). The assay was validated with two blinded sets of non-hemolytic and hemolytic drugs. Twelve of twelve clinically reported non-hemolytic drugs tested negative, and eight of nine hemolytic drugs tested positive in this assay, the exception being acetanilide. 8-AQ analogs have also been evaluated. Several agents that replenish intracellular reduced thiols and/or protect the cells from oxidant injury were tested for mitigation of hemotoxic effects of PQ metabolites. N-acetyl cysteine (NAC) has been reported to produce an increase in intracellular GSH, and decrease in oxidative stress. NAC partially attenuatee the hemotoxic effects of 5-hydroxyprimaquine (5-HPQ), a potential hemotoxic metabolite. A comparative evaluation of 5-HPQ and 8-N-hydroxy-6-methoxy-aminoquinoline (MAQ) showed differential hemotoxic responses. 5-HPQ produced about a 3- fold higher mtHb and more prominent depletion of GSH in G6PD-deficient human RBCs than MAQ; however, MAQ generated about 3-fold higher oxidative stress than 5-HPQ. In view of the structural similarities and oxidant potential of aminophenols (APs) and hydroxylated metabolites of 8-AQs, several AP analogs were evaluated *in vitro* for their hemolytic effects. The 2-APs generated markedly higher hemotoxic response compared to 4-APs, but 3-APs were non-toxic. 4-Methyl and 4-chloro substitutions potentiated the toxicity, while 4- and 5-nitro substitutions completely attenuated the toxicity of 2-APs. The results suggest possible structure-toxicity-relationships of APs and may be useful in designing new non-hemolytic 8-AQ analogs.

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PHENOTYPING PRIMAQUINE METABOLITES *IN VITRO* BY PRIMARY HUMAN HEPATOCYTES USING UPLC-QTOF-MS WITH STABLE ISOTOPE LABELING

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Primaquine (PQ) is an important antimalarial agent because of its activity against exoerythrocytic forms of *Plasmodium* spp. However, hemolytic anemia is a dose-limiting side effect of primaquine therapy that limits its widespread use in the clinic. The hemotoxicity is believed to be mediated by metabolites; however, the identity of the toxic species has remained unclear due to their highly reactive nature. The major plasma metabolite identified in humans and animals, carboxyprimaquine (cPQ), appears not to be responsible for this toxicity. Identification of minor metabolites in biological matrices poses a major challenge. Drug candidates labeled with stable isotopes in combination with LC/MS can be used to overcome this problem. This study was undertaken to identify the metabolites using UPLC-QTOF-MS from *in vitro* incubation of a 1:1 w/w mixture of $^{13}C_6$-PQ/PQ with primary human hepatocytes. An Acquity UPLC™ BEH Shield RP18 column (100 mm × 2.1 mm I.D., 1.7 µm) was used. The mobile phase consisted of water and acetonitrile, both containing formic acid at a flow rate of 0.25 mL/min with gradient elution. Acquity UPLC was integrated with QTOF-MS to combine the efficiency of separation with high sensitivity, selectivity of detection, and accurate mass. Qualitative metabolite identification was performed using MetaboLynx XS software. The lock mass compound was leucine enkephalin (*m/z* at 556.2771 and 278.1141). UPLC retention time, twin mass peaks with difference of 6 (originating from $^{13}C_6$-PQ/PQ), MS/MS fragmentation pattern, and percentage of metabolite (relative area% with respect to parent compound) were used for phenotyping and semi-quantitative analysis of metabolites. Besides cPQ, formed by oxidative deamination to aldehyde and subsequent oxidation, several other metabolites were identified: including PQ alcohol from oxidative deamination to aldehyde and subsequent reduction, the alcohol glucuronide conjugate and its acetate, as well as trace amounts of quinone-imine metabolites of PQ and cPQ, perhaps from hydroxylation at 5-position and subsequent oxidation.

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ANTIMALARIAL ACTIVITY OF METHYL JASMONATE AND EFFECT ON LIPID PROFILE OF *PLASMODIUM BERGHEI* INFECTED MICE

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Efforts at eradicating malaria has not yielded the desired results due to various challenges part of which is due to parasite resistance to commonly used antimalarial drugs. As part of the search for new antimalarial drugs, we screened methyl jasmonate (MJ), a fatty acid derived cyclopentanone and a component of the essential oil from flowers of *Jasminium grandiflorum* for *in vivo* activity in mice. *In vitro* study had indicated potential antimalarial activity of MJ. The Rane test procedure was used to assess the antimalarial activity of MJ. Forty-two BALB/C mice were infected with *P. berghei* NK65 (1 x 10^7) and divided into 6 groups. Groups 1, 2, and 3 received 10, 25 and 50mg/kg body weight of MJ respectively.

Groups 4, 5, 6 and 7 received chloroquine 10mg/kg, arteeether 3.2mg/kg, ethanol and normal saline respectively. All treatments were administered daily orally for four consecutive days. Thick and thin blood films were made from each mouse for 7 days and weekly for 28 days, stained with Giemsa stain and examined microscopically for parasitaemia. Twenty four hours after last administration, 3 mice from each group were sacrificed with serum used for liver function test and cholesterol, triglyceride, HDL and LDL determinations. Mean survival time were also documented. Methyl Jasmonates treatment resulted in a dose-dependent reduction in percentage parasitemia relative to control. 50mg/kg of MJ caused 54.4 % decrease in parasitaemia relative to chloroquine 81.3% and arteether 99.5% by Day 3. Mean survival time for 50mg MJ was 22.6 days compared with untreated (10-5 days), chloroquine (31.5 days) and arteether (27.2days). MJ like chloroquine and arteether treatment caused a marked decrease in cholesterol, triglyeride and HDL relative to untreated infected mice. There was a significant decrease in alkaline phosphatase MJ caused significant reduction in parasitaemia in a dose-dependent manner but less effective than chloroquine and arteether. MJ did not affect liver function enzymes and lipid profile adversely.

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NOVEL ANTI-MALARIALS: NAPTHOTHIAZOLIUM SALTS WITH POTENT ACTIVITY AGAINST *PLASMODIUM FALCIPARUM IN VITRO* AND *P. BERGHEI IN VIVO*

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Because of emerging resistance to existing drugs, novel classes of anti-malarial drugs with new mechanisms of action are needed. A large library of compounds was synthesized and designed to accumulate in the digestive vacuole of the malaria parasite and potentially catalyze the breakdown of hemozoin. Eight compounds in the original library were highly active against *Plasmodium falciparum in vitro*. The two most promising compounds are amphiphillic naphthothiazolium salts with amine-bearing side-chains. The most active compounds identified thus far are (1) KSWI-19855 which has an IC_{50} of 75nM against both chloroquine-sensitive and chloroquine-resistant *P. falciparum* (strains D10 and Dd2) and (2) KSWI-19854 which has an IC_{50} of 75nM against chloroquine sensitive *P. falciparum* (strain D10) and 0.5μM against chloroquine resistant *P. falciparum* (strain Dd2). In murine *in vivo* efficacy studies, both KSWI-19854 and KSWI 19855 demonstrate greater than 90% activity against *P. berghei* at 10mg/kg/day for 4 days. We postulate that these amphiphilic compounds reversibly enter the lipid nanospheres where hemozoin is synthesized inside the parasite food vacuole. Once in the food vacuole, we postulate that they depolymerize hemozoin by reducing the Fe^{+3} in hemozoin to its Fe^{+2} oxidation state, thereby breaking the iron carboxylate bonds holding the crystal structure together. Dose ranging studies and studies on the mechanism of action are on going. This project may lead to the clinical development of a desperately needed new anti-malarial drug.

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DEVELOPMENT OF SECOND GENERATION REVERSED CHLOROQUINE DRUGS

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Drug resistance is now seen against all of the approved antimalarial drugs. While eradication is the ultimate goal, currently there is still a need for new therapies to help those afflicted with this disease. We have previously reported on our 'Reversed Chloroquine' (RCQ) compounds, of which our lead candidate is undergoing preclinical testing. Here we present a structure-activity relationship (SAR) study designed to develop a 'second generation' candidate, to be ready in the event our primary drug stumbles on the preclinical road. Specifically, these next-generation RCQ molecules are designed to continue to improve the toxicity profile, while maintaining excellent *in vitro* and *in vivo* antimalarial activity.

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ANTIMALARIAL ACTIVITY AND TOXICITY OF 5 AND 7-METHYLATED PRIMAQUINE ANALOGS

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Primaquine, an 8-aminoquinoline derivative, is the drug of choice for radical cure of relapsing malaria caused by *Plasmodium vivax*, and is also used as a causal prophylactic agent against both *P. vivax* and *P. falciparum*. Primaquine in combination with clindamycin has also been shown to be effective for prophylaxis and treatment of *Pneumocystis carinii* pneumonia in AIDS Patients. A serious limitation to widespread use of this class of drugs, however, is that they produce reversible methemoglobinemia and hemolysis in individuals who suffer from hereditary glucose-6-phosphate dehydrogenase deficiency. Imino-quinone formed by oxidation of the 5- or 7-hydroxylated primaquine metabolite has been postulated to be responsible for this toxicity. If this mechanism is indeed involved, then substitution of a methyl group at 5 and/or 7- position in the quinoline ring of PQ can block the formation of the toxic metabolites. We prepared 5-, or 7-methylated, 5,7-dimethylated as well as 5-methoxy-7-methylprimaquine analogs and evaluated them for *in vivo* antimalarial activity in *P. berghei* mouse malaria model and *in vitro* methemoglobin formation in red cells incubated with the compounds in the presence of pooled human liver microsomes. Methyl substitutions at the 5 or 7 positions dramatically reduced the toxicity, but these analogs were also devoid of antimalarial efficacy. However, introduction of a methoxy group at the 5- position of primaquine improved the antimalarial activity but also increased its methemoglobin generating capacity in the *in vitro* assay. Introduction of 7-methyl group to 5-methoxyprimaquine greatly reduced both activity and toxicity. These results suggest that the blocking of activation of the 5 and 7 positions of the quinoline ring by methylation significantly reduces both toxicity and activity. These results will be discussed in light of the impact of other structural modifications that may improve the therapeutic window.

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LEAD OPTIMIZATION OF LIVER STAGE ACTIVE ACRIDONE ANTIMALARIAL

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Drugs targeting liver stage malaria offer many advantages in the prevention and eradication of the disease, but nearly all of the antimalarials currently in use or under development primarily act on blood stage infection. We have previously reported the discovery of a novel antimalarial acridone chemotype that displays efficacy against sporozoite-induced *Plasmodium* infection in addition to efficacy against the blood stage malaria. Significant improvement was achieved in the lead optimization process, and our latest lead candidate demonstrates potent efficacy in the following system: a) Prevention of *in vitro Plasmodium*

berghei sporozoite-induced development in human hepatocytes with an IC_{50} value of 2.2 ng/ml, comparable to that of atovaquone; b) Full protection from *in vivo P. berghei* sporozoite-induced liver stage infection in mice at 40 mg/kg/d (3X, oral doses); c) Low nanomolar inhibition of *in vitro P. falciparum* blood stage growth against a panel of multidrug resistant parasites; and d) Curative efficacy after oral administration against patent infection with *P. yoelii* in an erythrocytic murine model with an ED_{50} value of 1.2 mg/kg/d (3X), superior to chloroquine in the parallel study. Details of the design, chemistry, structure-activity relationships (SAR), safety, metabolic studies, and mechanism of action will be presented.

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EARLY-STAGE PRECLINICAL DEVELOPMENT OF REVERSED CHLOROQUINE (RCQ) HYBRID DRUGS

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We previously disclosed a class of molecules, termed Reversed Chloroquine compounds (RCQs), comprising a chloroquine (CQ)-like moiety linked to a Reversal Agent (RA) moiety, which reverses chloroquine resistance (CQR) in malaria. Structure-activity relationship (SAR) work has shown that the RCQ design is very flexible. We have constructed a substantial library of RCQ molecules that display *in vitro* efficacy - even sub-nanomolar IC50 values - against both CQR and CQS *Plasmodium falciparum*. The RCQ molecules have enhanced uptake, relative to CQ, into CQR parasites; they also diminish the activity of CQR-associated PfCRT protein mutants which have the ability to enhance efflux from the parasite's digestive vacuole. A subset of these drug candidates has been tested in mouse models of malaria, and found to be capable of reducing the parasite burden to below detectable limits - an oral cure. Both cytotoxicity and acute toxicity in mice are favorable, as is Ames evaluation of mutagenicity. SAR was applied to minimize hERG binding by the RCQ structures; an electrocardiogram study in guinea pigs to test for cardiac response shows a comparable response to that of CQ to high intravenous doses. Rat pharmacokinetics demonstrate good and tunable plasma levels and clearance times. A candidate RCQ drug has been selected and is moving through early preclinical studies.

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ENANTIOMERIC RESOLUTION OF 8-AMINOQUINOLINE ANTIMALARIALS

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Primaquine (PQ), an 8-aminoquinoline (8AQ) antimalarial agent, is the most prescribed drug for the treatment of relapsing malaria and is also an effective prophylactic agent against all plasmodia species. The major drawbacks of this drug are its short half-life and reversible methemoglobinemia and hemolysis in glucose-6-phosphate dehydrogenase deficient subjects. Studies during PQ development showed that the 4-amino-1-methylbutyl side chain on the 8-amino is prerequisite for optimum antimalarial activity. However, this side chain contains an asymmetric center, and conventional methods of preparation yield a racemic mixture. Tafenoquine, currently in clinical development, contains the same side chain and is also being developed as a racemate. Previous studies from our laboratory have shown that enantiomeric resolution of PQ into its individual enantiomers yields two analogs with markedly different efficacy, toxicity, and metabolism profiles. Enantioselective influences on efficacy and toxicity have also been observed with several other 8-AQ analogs with 5-aryloxy or 5-alkoxy substituents and the same 8-amino side chain. We have recently developed evidence that one enantiomer of NPC1161 (an analog with the same side chain) shows a 20-fold increase in efficacy over the other in a mouse causal prophylaxis model, but does not show a commensurate increase in hemolytic potential. In spite of the importance of this issue, it has been difficult to study individual enantiomers or to contemplate their economical development because of the lack of an efficient method to resolve them. A simple and generally applicable procedure has been applied to resolve different classes of 8-aminoquinolines as their phthalimides by fractional crystallization as diastereomeric salts with commercially available chiral organic acids. This procedure can be applied to resolve milligram to kilogram quantities, and affords a viable and economical option for development of new 8-AQ analogs.

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IMIDO-SUBSTITUTED NAPHTHOQUINONES: A NEW CLASS OF POTENTIAL ANTIMALARIALS

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The most dangerous form of human malaria is caused by *Plasmodium falciparum*, accounting for 80% of infections and 90% of deaths. Persistence of this disease in poorer countries of sub-Saharan Africa, Central and South America, and Asia represents a global crisis. Widespread resistance of *P. falciparum* to chloroquine and other commonly available antimalarial drugs exacerbates malaria mortality and intensifies the search for new drugs. Atovaquone, a hydroxynapthoquinone, is effective against multidrug-resistant parasites without *in vitro* evidence of cross-resistance. However, atovaquone is unsuitable for use as a single agent because of the relatively quick emergence of resistance. Several 1,4-napthoquinone derivatives originally investigated as antitumor drugs have been found to interact with novel targets, suggesting that this class of drugs may be effective against drug-resistant *P. falciparum*. For this study, imido-substituted chloro-1,4-naphthoquinone (IMDNQ) analogs have been synthesized and evaluated for antimalarial activity. Our hypothesis is that IMDNQ compounds will affect metabolic pathways distinct from those targeted by existing antimalarials and thus will be less susceptible to existing resistance mechanisms. IMDNQs were screened using a high-throughput malaria SYBR Green I assay. Of eight IMDNQs screened, four had IC50 values <10 ug/ml. Open chain IMDNQ analogs had higher antimalarial activity than cyclic IMDNQ analogs. Additional IMDNQ compounds, particularly open chain analogs, will be screened and the mechanism of action of lead compounds evaluated using a metabolomics approach. Once affected metabolic pathways are defined, evaluation of their direct target(s) and target:drug interactions will be used to further refine the structure of inhibitory compounds. Lead compounds will be evaluated against both drug-sensitive and -resistant parasites to evaluate their potential effectiveness against drug-resistant parasites.

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PROBING THE ANTIMALARIAL MECHANISM OF ACTION OF 1,2,4-TRIOXOLANES IN *PLASMODIUM FALCIPARUM*

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Artemisinin-based endoperoxides are highly potent, structurally complex trioxane antimalarials. Although ferrous activation of the endoperoxide bridge is considered key to drug activity, the mechanism of cytotoxicity remains elusive. Evidence supports a pathway whereby following iron

activation, endoperoxides form damaging free radical metabolites that target parasite macromolecules. Using fluorescent artemisinin analogs, we demonstrated endoperoxide-dependant labeling of neutral lipid bodies associated with the digestive vacuole. We proposed that localization of artemisinin metabolites was due to formation of covalent adducts that further initiated oxidative damage to parasite membranes, as measured by a free radical-sensitive BODIPY probe. A recently developed class of synthetic endoperoxides, comprising a 1,2,4-trioxolane flanked by cyclohexane and adamantane rings, show promise as potent and safe antimalarials. Here, we describe our efforts to similarly probe the localization and reactivity of the trioxolanes in *Plasmodium falciparum*. We applied trioxolane probes tagged with either an adamantane or cyclohexane dansyl group to living malaria parasites for observation by fluorescent microscopy. Our results show that iron activation results in molecular cleavage of the trioxolane producing an alkylating adamantane radical and a cytoplasmic cyclohexanone product. Labeling of neutral lipid bodies by the adamantyl portion of the trioxolanes was similar to that seen with the artemisinin analogs. Our collective findings using fluorescent trioxolanes suggests that endoperoxide-based compounds share a similar mechanism of action in malaria parasites that may involve targeting of neutral lipid bodies.

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A NOVEL CLADE OF EUKARYOTIC RIBONUCLEOTIDE REDUCTASE R2 SUBUNITS IS EXCLUSIVE TO APICOMPLEXAN PARASITES

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Apicomplexans are protist parasites of momentous public health and economic importance. The diseases they cause include malaria, cryptosporidiosis, East Coast fever, babesiosis and toxoplasmosis, and result in millions of deaths and billions of dollars in productivity and property losses each year. Research into new drug targets against these pathogens remains a high priority. Apicomplexan-related diseases may be controlled via inhibition of essential enzymes, provided that these proteins differ significantly in sequence or structure from homologs in their respective hosts. Ribonucleotide reductase (RNR) is one of 57 enzymes prioritized as potential drug targets against *Plasmodium*. RNR provides the only *de novo* means of synthesizing deoxyribonucleotides (dNDPs and dNTPs), the essential precursors for DNA replication and repair. While RNR has long been the target of antibacterial and antiviral therapeutics, targeting this ubiquitous protein to control eukaryotic pathogens may raise toxicity concerns due to its similarity to vertebrate RNR enzymes. The eukaryotic RNR holoenzyme is of the form $\alpha_n\beta_2$, and consists minimally of two large R1 and two small R2 subunits ($\alpha_2\beta_2$). We identified a novel clade of R2 subunits, R2_e2, which forms a sister group to the clade containing all eukaryote standard R2 subunits, R2_e1. Evidence suggests that R2_e2 subunits are functional and yet the amino acid sequence similarity between the two types of R2 subunits is <50%. Remarkably, while most eukaryotic genomes encode two standard R2_e1 proteins, apicomplexans encode one R2_e1 and one R2_e2. In fact, R2_e2 subunits have so far only been identified in apicomplexan genomes. Our results suggest that the novel R2 subunit unique to apicomplexans is a promising candidate for chemotherapeutic-induced inhibition, as it differs greatly from all known vertebrate RNRs and hence can potentially be specifically targeted.

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INTERPLAY BETWEEN COPY NUMBER VARIATION AND ANTIFOLATE RESISTANCE IN *PLASMODIUM FALCIPARUM*

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GTP-cyclohydrolase (*gch1*) is the first and rate-limiting enzyme in the folate biosynthesis pathway and has been found to exhibit extensive copy number variation in isolates from around the globe in areas with a history of longstanding use of antifolates. Specifically in South East Asia, increased *gch1* copy number is associated with increased likelihood of point mutations in dihydrofolate reductase (*dhfr*) and dihydropteroate synthase (*dhps*), genes which confer resistance to pyrimethamine and sulfadoxine respectively. One hypothesis for this finding is that an increased *gch1* is an adaptive response to compensate for less fit, drug resistant enzymes downstream in the folate pathway. To investigate the effect that *gch1* copy number has on antifolate-resistant parasites, we used a plasmid-based overexpression system, in which we can manipulate *gch1* copy number and expression levels in cultured parasites. We have implemented this system in multiple genetic backgrounds with different drug resistant profiles. We further tested whether the drug sensitivities of our parasite lines were altered using ^{3}H-hypoxanthine drug assays. Our results demonstrate that increases in *gch1* copy number and expression alter drug resistance phenotypes only in parasites bearing a mutant *dhfr*. This suggests that *gch1* amplification increases *dhfr* substrate concentrations relative to that of the inhibitor, thereby relieving the parasite of pyrimethamine pressure and rendering our current antifolate treatments inadequate. In addition, we have found that there is not a linear relationship between *gch1* copy number and expression levels in both isolates from around the globe and in our manipulated parasite lines which warrants further exploration. A greater understanding of the folate pathway and all the factors that play into the development of drug resistance is key to development of new drugs targeting this pathway and to understanding in general how the parasite can adjust to different drug pressures through both point mutations and copy number variation.

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WANING EFFECTIVENESS OF INTERMITTENT PREVENTIVE TREATMENT IN PREGNANCY (IPTP) WITH SULPHADOXINE-PYRIMETHAMINE (SP) IN THE PRESENCE OF HIGH SP RESISTANCE IN MALAWI

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Intermittent preventive treatment in pregnancy (IPTp) with sulphadoxine-pyrimethamine (SP) is recommended by the World Health Organization for the control of malaria in pregnancy in sub-Saharan Africa. Malawi was the first country to introduce IPTp with SP in 1993. Parasite resistance has compromised the efficacy of SP in the case-management of symptomatic children, but SP remained effective for IPTp in many areas of Africa. We conducted an observational study of women delivering in an area with high SP resistance (frequency of quintuple dhps/dhfr mutant haplotype >95%) in Blantyre district, Malawi to study the effect of SP resistance on

the efficacy of IPT-SP in preventing placental malaria and preterm delivery or low birth weight. Previous in-vivo studies in this area indicated that 50% of asymptomatic parasitaemic HIV-negative primi+secundigravidae (G1+2) who received IPT-SP were parasitaemic again within 42 days. Between Dec 2009 and Sep 2010, 780 HIV-negative women delivered (418 G1+2 and 362 multi-gravidae [G3+]), of whom 2.4%, 12.7%, 51.2% and 33.7% had received none, 1, 2, or 3 or more doses of IPTp-SP and 66.6% reported using a bednet. Among G1+2, the prevalence of placental malaria detected by histopathology or RDT was similar in each dose group (44%; 36%; 41%; 50% in the 0, 1, 2, 3 dose group respectively). Among G3+ the prevalence was lower among women receiving IPT, but there was no difference with each incremental dose (30%; 13%; 13%; 11%). The frequency of preterm delivery or LBW was similar in all dose groups among G1+2. Molecular analyses for SP resistance-associated mutations in dhps 436, 437, 540 and 581, dhfr 51, 59 and 164 and pfmrp1 1466 are ongoing and will be presented. These preliminary results suggest an absence of a beneficial impact of IPTp-SP among G1+2 protected by ITNs in this area with high grade SP resistance and near saturation of the quintuple dhps/dhfr haplotype. This raises concern about the longevity of IPTp-SP in southern Malawi and stresses the need to explore alternative drugs or strategies to replace SP or IPTp.

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MONITORING OF DRUG RESISTANCE AFTER INTERMITTENT PREVENTIVE TREATMENT FOR INFANTS AND CHILDREN (IPTI/C) IN SENEGAL

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In 2006, the health authorities of Senegal changed drug policy from sulfadoxine-pyrimethamine (SP)/amodiaquine (AQ) to the Artemisinin Combination Therapy (ACT) AQ/artesunate as first-line treatment against uncomplicated *falciparum* malaria. This was done due to reports of *Plasmodium falciparum* widespread resistance to SP and AQ. Currently, SP is still used for intermittent preventive treatment (IPT) as a method for reducing malaria morbidity and mortality and is being used in pregnant women (IPTp), infants (IPTi) and is being studied for children (IPTc). This study was undertaken to examine the impact SP use for IPTi and IPTc on the frequency of SP-resistant related haplotypes in the *Plasmodium falciparum* genes, Pfdhfr and Pfdhps. Samples were collected during a cross sectional survey in 2010 involving children under five years old living in three health districts located in the Southern Senegal where malaria transmission is high. Overall, 257 samples were *P. falciparum* positive. Among them, 176 individuals had received SP two years ago through IPTi in two of the districts while 81 did not. All positive samples were analyzed to determine the frequency of SP-resistance related haplotypes in Pfdhfr and Pfdhps based on results obtained by nested PCR followed by sequence-specific oligonucleotide probe (SSOP)-ELISA. The triple mutant Pfdhfr CIRNI haplotype dominated in both groups [IPTi+ (58%) and IPTi- (50%)]. The double mutant Pfdhfr CNRNI haplotype was also found with a frequency less than 5% in both groups. For Pfdhps, the wild type haplotype SAKAA dominated the control group with 28% (23/81) against 15% (26/176) with a significant difference (p=0.036). The double mutant Pfdhps haplotypes SGEAA and AGKAS were found in our study with a frequency less than 5% in both groups. The single mutant SGKAA haplotype was more frequent in IPTi+ group (30%) than in IPT- group (5%) the difference is not significant (p=6x10-6). In conclusion, the present study indicates that using SP for IPTi does not select resistant parasites when follow up is performed long term. Base on WHO recommendation, SP can still be use as IPTi in Senegal because of the very low frequency of Pfdhps haplotype SGEAA (<5%)

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THE RETURN OF WIDESPREAD CHLOROQUINE SENSITIVE *PLASMODIUM FALCIPARUM* TO MALAWI

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Since chloroquine-resistant *falciparum* malaria became pervasive in Africa, the reemergence of predominantly chloroquine-sensitive parasite populations has been documented in Blantyre, Malawi, an urban center in Eastern Africa. This resurgence of sensitive parasites followed a change in national treatment policy from chloroquine to sulfadoxine-pyrimethamine in 1993, and treatment efficacy of 99% was demonstrated in 2005. Studies in other areas of Malawi report varying results on resistance levels outside of this population center. This study evaluated the prevalence of chloroquine drug resistance using a marker in the *Plasmodium falciparum* chloroquine resistance transporter *(pfcrt)* gene throughout the country, including rural areas and districts bordering countries where chloroquine use persisted much later than 1993. Dried blood spots were collected from children aged five years or less using two-stage cluster sampling in eight districts across Malawi in 2009. Samples with *P. falciparum* parasitemia on microscopy underwent PCR amplification and pyrosequencing of the *pfcrt* gene 76 amino acid region to determine chloroquine resistance status. Of 7145 samples collected, 1168 were found to have parasitemia by light microscopy. Of 696 with sufficient DNA for sequencing only 2 were found to have the chloroquine resistance genotype. This translates to an overall proportion of infections with detectable resistance of 0.003 (95% CI:-0.001,0.007) and a proportion of 0.167 (95% CI: -0.262,0.595) in Karonga and 0.007 (95% CI: -0.007, 0.020) in Mwanza, the two districts where resistant samples were found. Sampling over a wide geographic region of Malawi, including higher risk sites for ongoing resistance such as border areas indicates that chloroquine-susceptible malaria now predominates the parasite population in this country. A very small subpopulation of resistant parasite nevertheless appears to persist within this population, suggesting that resumption of chloroquine use might be quickly followed by selection and increasing prevalence of chloroquine-resistant parasites.

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A NETWORK-BASED APPROACH TO PROBING THE METABOLIC PATHWAYS OF *PLASMODIUM FALCIPARUM*

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There is a growing demand for high resolution data to quantify and characterize the enzymic and metabolitic status of the human malaria parasite, *Plasmodium falciparum*. The resolution of a metabolic network offers insights into the life cycle and pathophysiology of the parasite. Since metabolites are the ultimate cellular readout, investigating the

global metabolic flux regulation of an organism is generally more informative than measuring mRNA levels. Our approach is enhanced by the incorporation of network theory and graphs that model the interconnected and sequential conversions of compounds in metabolic pathways. The profile of individual metabolite levels inherited in progeny of a genetic cross can serve as a phenotype to uncover genetic factors underpinning parasite physiology using quantitative trait loci (QTL) mapping. We extracted metabolites from the parents and progeny of HB3 × Dd2 and 7G8 × GB4 genetic crosses of *P. falciparum* at three erythrocytic cell cycle stages and constructed a metabolic network using Pearson's correlation of metabolite levels obtained from LC-MS. Individual mass signatures, the vast majority still unidentified, map to all chromosomes in the genome in an asymmetrical manner such that a few loci influence the levels of many compounds while other loci affect none. Our network approach does not rely on QTL; however, the network modularity of clustering patterns of compounds can be used to evaluate the significance of QTL. We investigate whether co-mapping compounds from QTL hotspot regions also cluster together in the network. Finally, the network provides an interpretive framework for the prioritization of these unknown compounds by clustering metabolites involved in specific pathways and by leveraging information about the known metabolites. These studies establish a framework to construct and analyze the metabolite network in *P. falciparum*, and will ultimately provide useful insights about antimalarial drug resistance and prospective targets.

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GAMETOCYTE CLEARANCE DYNAMICS FOLLOWING ORAL ARTESUNATE TREATMENT OF UNCOMPLICATED *FALCIPARUM* MALARIA IN MALI, WEST AFRICA

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Artemisinin-based combination therapies (ACTs) reduce *Plasmodium falciparum* gametocyte carriage, but their true effect on gametocytes and on transmission potential is not fully understood. A better understanding of gametocyte dynamics *in vivo* in the presence of artemisininsis needed. One hundred children aged 1-10 years presenting with uncomplicated *falciparum* malaria to a sentinel site clinic in Bougoula-Hameau, Mali were treated with seven days of directly-observed oral artesunate therapy from December 2010 to February, 2011. Thick and thin blood smears were prepared and read every 8 hours until three consecutive slides were negative for asexual *falciparum* parasites. Gametocytes were quantified by two trained microscopists using standard WHO procedures. Gametocyte carriage and density were compared at 0, 1, 2, 3, 7, 14, 21 and 28 days after treatment initiation using the chi-square test and the student's t-test, respectively. Of 92 children in the final analysis, 21 (22.83%) were gametocyte carriers at the time of treatment initiation. The proportion of gametocyte carriers was unchanged at the end of treatment (day 7, 23.91%, p=1.0) and did not significantly decline until day 21 of follow-up (6.52%, p=0.003). The mean gametocyte density at inclusion, 11.78 gametocytes/µl, also remained unchanged at the end of treatment (13.25 gametocytes/µl, $p > 0.05$) and only dropped significantly at day 28 of follow-up (0.62 gametocytes/µl, p=0.01). Among carriers at inclusion, the median clearance time was 14 days. Among non-carriers at inclusion, 6 (8.11%) became carriers by day 7. Artesunate decreased gametocyte carriage and gametocyte density by the end of the 28-day follow up. However, artesunate did not prevent the maturation of young gametocytes to circulating stage V, as evaluated by standard microscopy. More sensitive gametocyte detection methods may better characterize these dynamics. Further work is needed to determine the role sequestered gametocytes may play in the persistence of peripheral gametocytemia after artemisinin-based treatment initiation.

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RESEARCH CAPACITY DEVELOPMENT FROM SCRATCH: THE EXPERIENCE OF THE WEST AFRICAN NETWORK FOR CLINICAL STUDIES OF ANTIMALARIAL DRUGS (WANECAM)

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Malaria remains a major public health problem in much of sub-Saharan Africa. Yet there is little data on the epidemiology, transmission and drug resistance in many areas of the Continent. To address these issues in Guinea, West Africa, we are building human capacity, infrastructure and the regulatory frame work necessary for conducting state of the art clinical research. A TDR initiative of 1997 selected a young Guinean Scientists with strong potential for research. The scientist received nearly 8 years of training in the laboratory and in the field in France and in Mali leading to a MSc and a PhD degrees in Parasitology. He was then invited to join the EDCTP funded WANECAM project. A site assessment visit by senior members of the Network helped in streamlining the needs in human capacity, infrastructure and regulatory environment. A team of 8 young scientists with little or no experience in research was recruited. The team received intensive short-term training in GCP, ethics, computer skills and clinical studies. Training included short-term workshops both in Guinea and abroad, the posting of experienced Malian scientists in Guinea for extended periods and short visits by experienced senior staff from the other network members. Two students were enrolled for MSc training in Burkina Faso and in Mali. Two 4-weel drive vehicles were purchased. A vacant building was obtained from the Government of Guinea and refurbished into a brand new polyvalent laboratory. As a result, the first malaria entomology survey was conducted. A prospective longitudinal study on references ranges of biological parameters, age specific incidence and drug resistance is underway. A solid and emerging malaria research team is now in the building in Guinea. This experience underlines that capacity development in developing countries is a long-term investment on the scientists, the environment, and the physical infrastructure.

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EFFICACY OF ARTEMETHER-LUMEFANTRINE AND ARTESUNATE-AMODIAQUINE FOR THE TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* INFECTION IN TANZANIA

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Following the development of drug resistance to anti-malarial first line treatment of uncomplicated malaria with sulfadoxine-pyrimethamine (SP) by *Plasmodium falciparum* in mainland Tanzania the ministry of health and social welfare (MOHSW) introduced artemisinin combination therapy (ACT) with artemether-lumefantrine (ALu) as first line treatment for the treatment of uncomplicated *falciparum* malaria in 2006. There

is growing evidence suggesting that malaria cases over the past three years and entomological inoculation rates (EIR) that are currently monitored in most parts of Tanzania are declining. Despite good malaria control achievements, there is a threat of ACT drug resistance. Due to recent report on the emerging drug resistance to ACT along the Thai-Cambodia border it is critical to our region to monitor the spread of drug resistance to ACT. We set up to conduct an invivo monitoring study at four country-wide representative National Malaria Control Programme (NMCP)'s sentinel sites in May-August 2011 to assess efficacy of ALu and amodiaquine-artesunate both anti-malaria first line in Mainland Tanzania and Zanzibar respectively. The study sites are Mlimba, Mkuzi, Kibaha, and Muheza in the mainland Tanzania. Participants are febrile patients aged 6-59 months presenting at the health facility to be followed up during 28 days to elicit treatment performance. Results of this study will be out by the time of American Society of Tropical Medicine and Hygiene conference in November 2011. We will elucidate the occurrence of drug resistance by PCR using msp1 and glurp. As some of the current molecular genotyping malaria tools are based on SP which is also used for chemoprophylaxis (IPTp or IPTi) we will also generate data on molecular markers (dhfr and dhps) for SP resistance. This analysis will assist to monitor the evolution, spread and intensification of ACT and SP resistance. Results from this study will be used to assist the MOHSW to assess the current national treatment guidelines for uncomplicated. *Falciparum* malaria.

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EFFICACY OF FIXED-DOSE COMBINATION ARTESUNATE-AMODIAQUINE VERSUS ARTHEMETHER-LUMEFANTRINE FOR UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN CHILDREN UNDER FIVE: A RANDOMIZED NON INFERIORITY TRIAL IN DEMOCRATIC REPUBLIC OF CONGO

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Until now, only a limited number of studies have been published in Central Africa measuring the efficacy of artemisinin combination therapies (ACTs) since their introduction. The Democratic Republic of Congo (DRC), one of the largest countries in the region, adopted artesunate and amodiaquine (ASAQ) as first line antimalarial treatment in 2005. We conducted a randomised open-label non-inferiority trial, enrolling children aged 6-59 months with uncomplicated *P. falciparum* malaria in Pweto district, Katanga province. Patients were randomly allocated into one of the two regimens, fixed-dose formulation ASAQ or artemether-lumefantrine (AL). We analyzed the risk of recurrent parasitemia by day 42 adjusted by PCR genotyping, expressed as estimates of failure from survival analysis and as simple proportions (per protocol). Of 1993 children who were referred to the study clinic between April 2008 and March 2009, we enrolled 301 children: 156 with ASAQ and 145 with AL. The proportion of patients with parasitemia were low in both groups at D2 and D3: 6.0% (9/150) in the ASAQ arm and 4.9% (7/143) in the AL arm; and 0.6% (1/150) and 0.7% (1/143) respectively. After PCR correction, cure rates were 98.3% (95%CI, 94.1-99.8) in the ASAQ group and 99.1% (95%CI, 94.9-99.9) in the AL group (difference -0.7%, one sided 95%CI -3.1). Kaplan-Meier PCR-adjusted cure rates were similar: ASAQ, 98.4% (95%CI, 93.8-99.6) vs AL, 99.2% (95%CI, 94.3-99.9). Both treatment regimens were well tolerated. The results show that ASAQ was not inferior to AL and that both ACTs were highly effective as first-line malaria treatment in this area. The logistical constraints of a remote site and the slow recruitment of comfirmed cases were among the main challenges and increased substantially the cost of the study. The recommended therapeutic efficacy surveys throughout the territory at repeated intervals are difficult to achieve considering the logistical challenges and the limited technical capacity in a country like DRC.

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MONITORING THE EFFICACY AND SAFETY OF ACTS TO TREAT UNCOMPLICATED MALARIA IN BOBO-DIOULASSO, BURKINA FASO

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Malaria in Burkina remains the major public health compromising therefore the development of the country. Since 2005, the national malaria control program advocated artéméther-lumefantrine (AL) and artesunate-amodiaquine (AS-AQ) respectively as first and second lines for the treatment of uncomplicated *falciparum* malaria. Monitoring efficacies of these artemisinin based combination therapies play a major role in early detection and containment of resistance. We compared efficacies of AL and ASAQ for the treatment of uncomplicated *falciparum* malaria in two randomized trials with patients aged over 6 months. Outcome of treatment were defined according to standard WHO classification, ETF, LCF, LPF and ACPR. Genotyping to distinguish recrudescence from new infections is ongoing. Overall, 618 patients included in both studies completed their follow-up. We did not noted any ETF and at day 28, risk of recurrent infection were 9/66 (13.6%) in AL group compared to 4/62 (6.5%) in ASAQ group in 2009 and 46/211 (21.8%) compared to 20/215 (9.3%) in 2010. Most of treatment failures were new infections and PCR corrected ACPR were similar for both drugs in the two studies. No serious adverse event related to the studies drugs was recorded. Known polymorphisms-mediating resistance in pfcrt and pfmdr1 were not associated with treatment failure. All study drugs have shown excellent efficacy and safety in treating uncomplicated *falciparum* malaria in Burkina but the concern might be the reported resistance-mediating polymorphisms selection by the partner drugs following treatment.

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ESTIMATING SELECTION ON *PLASMODIUM FALCIPARUM* DRUG RESISTANCE ALLELES IN AN ENDEMIC POPULATION OVER A 25-YEAR PERIOD

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Using archived blood samples, we surveyed the changes in drug resistance alleles in The Gambia over a 25 year period from the time when resistance was unknown locally (in 1984) through periods of gradual failure of chloroquine therapy and increasing use of sulphadoxine-pyrimethamine until eventual introduction of artemisinin combination therapy (in 2008). At the first survey there were no drug resistance alleles detected at two of the loci (*crt* and *dhps*) and very few isolates contained resistance alleles at the other two loci (*mdr1* and *dhfr*). Proportions of isolates with resistance alleles increased progressively over subsequent surveys, reaching peaks for the chloroquine resistance alleles *crt* 76T (76%) and *mdr1* 86N (78%) in the year 2000, and for antifolate resistance *dhfr* alleles (94%, mostly as a triple combination of 51I, 59C and 108N) and *dhps* 437G (86%) in 2007 and 2008 respectively (the *dhps* resistance allele 540E was not present in any of 623 isolates genotyped over the whole period). To estimate changes in allele frequencies over time, we counted one allele at each locus per isolate, randomly sampling when there were mixed genotypes, and estimated 95% confidence intervals based on sample sizes in each year. Changes in allele frequencies occurred at different times and rates over the period of survey, and the data fit closely a very simple model for each locus with assumed fitness costs and a change in selection

coefficients reflecting historical change in therapeutic use. We explore the fit between these historical selection data and signatures of selection at these loci that can be derived from genome wide polymorphism data in a population sample taken at the end of the period.

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ALIGNMENT AND GENE SET ENRICHMENT ANALYSIS OF TIME-COURSE PROFILES OF RECOMBINANT PFMDR1 AND PFCRT-MODIFIED *PLASMODIUM FALCIPARUM* PARASITE LINES

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Transcriptional profiling studies of the intraerythrocytic developmental cycle (IDC) of *Plasmodium falciparum* have revealed a unique transcriptome characterized by a continuous cascade of expression. Here we present a quantitative time-course analysis of the gene expression levels of 6 strains that differ in 2 key antimalarial resistance determinants, pfmdr1 and pfcrt. Continuous expression profiles were imputed from the 8 time points sampled for each strain and then aligned through dynamic time warping. Transcriptional differences were elaborated at both the gene and gene set level using a novel algorithm that measures gene set enrichment at many discrete time points along the imputed and aligned expression profiles. Significantly up or down-regulated gene sets were identified in each comparison along with the time period of maximal enrichment. We present software to visualize the complete aligned expression profiles of each strain in 2 or 3 dimensions, facilitating comparison of individual time points as well as the full time-series. Comparison of our alignment methods with conventional techniques underscores the vital role that temporal alignment plays in discriminating genuine biological signal from the transcriptional noise created by gene expression cascades peaking at different time points and durations. Together, our data and software tools provide a window into the rich transcriptional complexity of *P. falciparum* parasites by allowing the alignment and comparison of strains that differ in fitness and therefore progress through the IDC at varying rates.

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THE INTERACTION BETWEEN MALARIA PARASITES AND BLOOD GROUPS IN PORT HARCOURT, NIGERIA

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The impact of malaria on the public health of resource-limited economies of the world is still a major problem particularly in Africa where 89% of all malarial deaths occur. The pathogenesis of *Plasmodium* infection entails merozoite invasion of erythrocyte, which implies an implicit interaction between the red cell membrane proteins and the invading plasmodium antigens. Blood group antigens serve as genetic markers of several clinical conditions including malaria; the clearest example being the well elucidated inter-relationship between *Plasmodium vivax* and the Duffy antigen. Thus, the products of research on blood groups and malaria may have a potential impact on the development of new anti-malarial chemotherapy, vaccines and reduction of the global burden of malaria. This study was designed to investigate the link between blood groups and different malaria parasites in Port Harcourt, Nigeria which is the centre of the oil and gas industry in West Africa. Furthermore, we will investigate the incidence of *Plasmodium ovale* and the specificity of the parasite strain in relation to various blood groups in this environment. Thick blood smears and filter paper blood spots were made from finger-prick for microscopy and molecular genotyping of parasite strains. Two hundred and forty six participants: 142 males (57.72%) and 104 females (42.28%) aged 16-60 years attending the Braithwaite Memorial Hospital and blood donors presenting at the University of Port Harcourt Teaching Hospital Blood Bank were enrolled into the study. Preliminary results showed that 207 (84.1%) were positive for *Plasmodium falciparum* while 39 (15.9%) were negative by microscopy. However prevalence of other species is expected from the PCR genotyping. Results of the blood group screening showed that blood group O Rh positive was the highest with 163 (66.2%) followed by blood group A Rh positive 43 (17.5%), B Rh positive 26 (10.6%), O Rh negative 7 (2.85%), AB Rh positive 5 (2.03%), B Rh negative 1 (0.41%) and A Rh negative 1(0.41%).

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BASOPHIL REACTIVITY IS ASSOCIATED WITH MALARIA SEVERITY AND PFTCTP

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Understanding of malaria immune-pathogenesis will lead to the identification of new therapeutic strategies aimed at improving recovery. Recent findings have suggested common mechanisms in malaria pathogenesis and allergy. Elevation of IgE levels has been associated with malaria infection, but their role remains unclear. Similarly, a parasite-derived histamine releasing factor (PfTCTP) was found at high level in serum from patients but *in vivo* effects are unknown. To address these questions, we conducted a clinical study in Dakar (Senegal). *Plasmodium falciparum* infected patients with mild (MM, n=19) or severe (SM, n=9) symptoms were enrolled and compared with healthy controls (HC, n=38). We performed basophil activation tests on whole blood samples based on CD203c expression to analyse allergic response. Basophils from MM patients showed significant lower baseline levels of CD203c expression, compared to SM and HC. Basophils from SM patients were characterized by a higher reactivity to A23187, haemozoin and anti-IgE stimulation. Ex vivo priming of basophils with recombinant human or PfTCTP before stimulation with anti-IgE induced either an enhancement or an unexpected decrease in activation (mostly in MM and HC patients). The decrease in basophil activation, previously described as an "overstimulation", suggests a better ability of HC and MM patients to control allergic response following excessive stimulation. IgE levels were also higher in malaria patients than in healthy ones, but were not related to basophil responses. Indeed the reactivity of basophils from malaria patients was positively related to the presence of circulating PfTCTP or for SM, to the lack of anti-PfTCTP IgG. Altogether these data revealed a high reactivity of basophils during SM which could explain the high level of histamine reported during SM, likely contributing to blood-brain-barrier impairment. These findings support an involvement of allergic immune responses in malaria pathogenesis which can be exacerbated by the proinflammatory environment and PfTCTP.

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IPT/C: PREVALENCE OF ANTIBODY AMA-1 AND MSP-1(19) IN THREE AREA IN SENEGAL

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Malaria remains a major disease in many African countries. Nowadays, many strategies such as IPTic /SP are used for prevention in children. But SP- resistant parasites can compromise this strategy. To evaluate the impact of IPTic /SP on antigenic variation in rural areas of three districts, all children aged 5mths-10years, in Senegal. In 2009, to assess the role of

serological markers in evaluating malaria transmission, filter papers were collected from children under 10 years. Filter blood spot papers were collected from 5833 people from Mbour, Bambey and Fatick to assess the prevalence of antibodies to two *Plasmodium falciparum* antigens MSP-1(19) and AMA-1. Seropositivity to *P. falciparum* MSP-1(19) was 15.5 % and 26.7% to AMA-1. MSP-1(19) is lower than AMA-1. Fatick presents most of positive children who answer to antibody. Also in Fatick the young children have least antibody. Seroepidemiology can provide key information on malaria transmission for control programmes, when parasite rates are low.

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COMPARATIVE PROTEOMIC ASSESSMENT OF *PLASMODIUM CHABAUDI ADAMI* AS-INFECTED AND NAÏVE MOUSE SERUM TO IDENTIFY CANDIDATE CFF PROTEINS

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The identity of serum crisis form factor (CFF) has remained elusive for decades after its initial characterization as a factor in human immune sera able to inhibit growth and cause the intraerythrocytic degradation of the malaria parasite, *Plasmodium falciparum*, in culture. CFF is named for the association of its coincident presence with the immunological crisis leading to resolution of infection and is inducible through artificial immune stimulation in rabbit and rodent models by treatments such as BCG/LPS and malaria infection. CFF has been described in the serum of some individuals with apparent resistance to malaria symptoms and may provide a novel mechanism of natural immunity. Identification of CFF would enhance our understanding of how immune system components interact with a *P. falciparum* infection to produce acquired immunity, which is a critical component of the current investigation into a malaria vaccine. In this study, we used the C57BL/6 mouse model inoculated with *P. chabaudi adami* AS to induce serum CFF, as documented by inhibition of *P. falciparum* growth in culture and the appearance of classic CFF responses in microscopic findings. We isolated the low abundance protein fraction of these CFF mouse sera using IgY depletion. Proteomic analysis using MALDI and LC-QToF was conducted on depleted serum from the C57BL/6 *P. chabaudi adami* AS model and non-inhibitory serum from naïve mice. Protein differences were quantified to discover proteins that were present in the CFF serum and absent from naïve serum. This analysis highlighted 68 proteins as either up-regulated or unique to CFF serum, and a qualitative analysis revealed potential CFF candidates. This study provides new insight into the etiology of CFF and host serum protein changes during a malaria infection.

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PEDIATRIC MALARIAL ANEMIA SEVERITY IS DEFINED BY ELEVATED LEVELS OF CIRCULATING MEMORY CD4 T CELLS PRODUCING IL-17

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In *Plasmodium falciparum* holoendemic transmission regions of western Kenya, severe pediatric malaria manifests as severe malarial anemia (SMA). We hypothesized that children presenting with SMA would have chronic immune responses characterized by effector/central memory CD4+ T cells producing interferon (IFN)-γ and/or interleukin (IL)-17 that suppressed their erythropoietic responses. We therefore characterized the CD4+ T cell populations and their intracellular IFN-γ and IL-17 production in healthy controls [HC; hemoglobin (Hb)≥11.0g/dL, without parasitemia, n=13] and febrile children with differing levels of malarial anemia severities and any density parasitemia: uncomplicated malaria (UM, Hb≥11.0g/dL, n=140; mild malarial anemia (M/MA, Hb≥8.0≤10.9g/dL, n=23); and SMA (Hb<6.0g/dL, n=23). Across group comparisons revealed that children with SMA had elevated effector memory (T_{EM}) (CD4+CD62L-IFN-γ+) cells [median (IQR) 92.60% (7.50)] relative to the HC [75.00% (19.10)], UM [62.80% (15.50)], and M/MA [66.70% (25.00), *P<0.001*] groups., T_{EM} (CD4+CCR7-IL-17+) cells were also highest in the SMA group [87.15% (5.80)] compared to the HC [44.80% (15.40)], UM [58.05% (13.40)], and M/MA [78.40% (10.20), *P*<0.001] groups. In addition, the SMA group had higher integrated mean fluorescence intensity (iMFI) for IFN-γ in T_{EM} cells [HC, 1628.48 (719.60); UM, 1521.31 (852.20); M/MA, 1994.33 (1397.50); and SMA, 3429.30 (1758.20) (*P*<0.001)]. The iMFI of IL-17 in T_{EM} cells increased with disease progression towards SMA [HC, 1386.00 (1293.70); UM, 1895.00 (634.70); M/MA, 2716.55 (2567.30); and SMA, 5718.80 (1540.1), *P*<0.001]. Moreover, the iMFI of IFN-γ and IL-17 in T_{EM} cells were negatively correlated with Hb levels (r=-0.600, *P*<0.001; and r=-0.690, *P*<0.001, respectively). Our findings suggest the involvement of T_{EM} producing IFN-γ and/or IL-17 in pediatric SMA pathogenesis.

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IDENTIFICATION OF HOST TRANSCRIPTIONAL PROFILES ASSOCIATED WITH ASYMPTOMATIC MALARIA AFTER A BOUT OF SEVERE MALARIA

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Clinical signs of *Plasmodium falciparum* can range from cerebral malaria to asymptomatic carriage. Prior exposure and host genetics will alter clinical presentation; however, the mechanisms associated with clinical presentation are not fully characterized. To explore the role of the host response across clinical phenotypes, we studied human whole genome transcription expression profiles from children with cerebral malaria admitted to the Blantyre Malaria Project during a single malaria season. Survivors are invited to return for a one-month follow-up visit, and we analyzed samples from survivors found to have asymptomatic malaria infections at that time. Whole blood (2-3 mL) was collected, stabilized in Tri-Reagent, and frozen at -80°C at the time of admission and at follow up (day 30). RNA was isolated from sixty severe disease samples and five follow-up matched samples. RNA was hybridized to Affymetrix GeneChip Human 1.0 ST Arrays. For the paired analysis of the severe and asymptomatic samples (n=5), we identified significantly differential gene sets using GSEA (GenePattern, Cambridge, MA) software. The severe disease presentation in the matched samples was significantly associated with olfactory sensory transcripts (GO:olfactory sensory receptor activity). The olfactory bulb is unique to the central nervous system in that it has an external component. We speculate that our peripheral blood analysis may be detecting this peripheral component of the brain, reflecting the central nervous system abnormalities involved in cerebral malaria. GO sets significantly upregulated in samples derived from the asymptomatic presentation reflect immune system upregulation (GO:regulation of the immune system processes; GO:regulation of leukocyte differentiation). This is the first report that captures the peripheral blood transcriptomes during a bout of severe malaria and during a subsequent asymptomatic infection

and may provide insight into host response associated with clinical presentation to inform pathogenesis/immunity models and potential targets of intervention.

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CD11C EXPRESSION DEFINES MULTIPOTENT EFFECTOR MEMORY CD8 T CELLS INDUCED BY GENETICALLY-ATTENUATED MALARIA VACCINES

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Vaccination with live, genetically-attenuated *Plasmodium yoelii* parasites (PyGAPs) can induce long-lasting sterile protection against liver stage malaria in mice with just one dose. The underlying mechanisms mediating this protective immune response are not fully understood, but further characterization will be vital for guiding future vaccine design. Previous work from our lab demonstrates that protective immunity following PyGAP immunization is completely dependent on CD8 T cells, partially dependent on IFN-γ and perforin, and likely mediated by direct cytotoxic killing of parasite-infected hepatocytes. In addition, protective efficacy correlates with expansion of effector memory CD8 T cells in the liver. We went on to further characterize vaccine-induced changes in the T cell phenotype and found significant up-regulation of CD11c on CD3+CD8+ T cells in the liver, spleen and peripheral blood. As much as 50% of CD8 T cells co-expressed CD11c in the liver, which is the site of infection, and expansion of the CD11c+ CD8 T cell population correlated with protective efficacy following various vaccine regimens. CD11c expression was specifically induced on T cells from immunized mice but not from control mice following co-culture with malaria-infected hepatocytes. Further analysis demonstrated that these CD11c+ T cells are predominantly CD11a+ $CD44^{hi}$ CD62L-, indicating that they are antigen-experienced, effector memory cells. Following *in vitro* re-stimulation with malaria-infected hepatocytes, CD11c+ CD8 T cells expressed multiple inflammatory cytokines and cytotoxicity markers, including IFNγ, TNFα, IL-2, perforin and CD107a. CD11c- CD8 T cells, on the other hand, expressed negligible amounts of inflammatory cytokines and cytotoxicity markers, indicating that CD11c expression accurately defines multifunctional effector CD8 T cells. Surprisingly, we found that CD11c+ CD8 T cells also express other antigen-presenting cell (APC) markers, including MHC class II, CD80 and CD86, suggesting that these cells may have an unusual APC-like phenotype. Taken together, our data demonstrate that CD11c+ CD8 T cells are multipotent effector memory cells that are likely to mediate the protective immune response against liver stage malaria infection following PyGAP vaccination.

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DECREASED SYSTEMIC PROSTAGLANDIN (PG)-E_2 AND CYCLOOXYGENASE (COX)-2 GENE EXPRESSION IN CHILDREN WITH SEVERE MALARIA ANEMIA AND CO-INFECTION WITH HIV-1 OR BACTEREMIA

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In malaria endemic regions of western Kenya, *Plasmodium falciparum* malaria manifests clinically as severe malarial anemia [SMA; hemoglobin (Hb)<6.0g/dL, any density parasitemia]. Although we have previously shown that prostaglandin (PG)-E_2, cyclooxygenase (COX)-2 transcripts and protein levels are reduced in children with severe and cerebral malaria, the impact of HIV-1 and bacteremia co-infections, and *in vivo* malaria pigment containing monocytes (PCM) on systemic PGE_2 production and COX-2 mRNA expression in children with SMA has not been investigated. As such, we investigated plasma and urine PGE_2 (measured as bicyclo-PGE_2) and COX-2 mRNA expression in children with clinical malaria (n=74) and those co-infected with either HIV-1 (*Pf*+/HIV-1+; n=8) or bacteremia (*Pf*+/bacteremia+; n=19). Plasma (P=0.001) and urinary (P<0.001) PGE_2 levels were decreased in children with SMA relative to the non-SMA (Hb≥6.0g/dL, any density parasitemia) group. Additionally, PGE_2 levels were lower in *Pf*+/HIV-1+ children in plasma (P<0.001) and urine (P=0.007), as well as *Pf*+/bacteremia+ children in plasma (P<0.001) and urine (P=0.173), relative to those with malaria infection alone. PGE_2 increased with increasing hemoglobin levels in children with malaria (plasma; r=0.363, P=0.002 and urine; r=0.500, P=0.001), and in co-infected children (*Pf*+/HIV-1+; r=0.819, P=0.013 and *Pf*+/bacteremia+; r=0.595, P=0.007). Additional analyses demonstrated decreasing PGE_2 levels with increasing PCM in plasma (P=0.031) and urine (P=0.070). COX-2 mRNA expression was decreased in children with SMA relative to the non-SMA group (P=0.011) and in *Pf*+/bacteremia+ (P=0.033) and *Pf*+/HIV-1+ children (P=0.118) relative to those with malaria alone. Taken together, results demonstrate that SMA is associated with decreased PGE_2 and COX-2 gene expression, and is further augmented by co-infections (HIV-1 and bacteremia), driven in part, by naturally acquired malarial pigment by monocytes.

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LIVER-RESIDENT CD8+ T CELLS INDUCED BY RADIATION-ATTENUATED *PLASMODIUM* SPOROZOITES

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Memory CD8+ T cells induced by malaria sporozoites home to the liver and eliminate parasite-infected hepatocytes. While memory T cells residing in lymph nodes, spleen, lung and peripheral blood are polyfunctional, capable of mediating cytotoxicity and producing multiple cytokines, the liver-resident memory cells exhibit a unique monofunctional profile with normal cytotoxic activity but minimal cytokine production. This phenotype is specifically induced by parasites but not viruses expressing the same epitope. The liver-resident memory cells are not exhausted, anergic or senescent, albeit their proliferation after *in vivo* antigen re-exposure is markedly reduced. Importantly, these cells undergo vigorous homeostatic proliferation, display normal *in vivo* cytotoxic activity and inhibit parasite development in hepatocytes. Surprisingly, these cells are fully capable of producing IFN-γ transcripts but translation occurs only in response to TCR-independent stimuli. These results suggest that parasite-induced liver-resident memory CD8+ T-cells represent a distinct terminal effector lineage characterized by a monofunctional profile maintained in part through translational control of cytokine production.

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USING PROTEIN ARRAYS FOR ANTIBODY PROFILING AND DISEASE STRATIFICATION IN MALARIA INFECTION

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The prevalence of mixed-species malaria infections was underestimated until more sensitive detection methods, such as PCR-based diagnosis, were introduced for epidemiological studies in malaria endemic areas. In the era of malaria elimination, improved diagnostic tools are required to enable targeted treatment of infected individuals as well as effective mass screening for the detection of very low parasite densities to monitor

transmission reduction. Serological markers represent a promising tool for diagnostics and surveillance, especially for *Plasmodium vivax*, for which current rapid diagnostic tests are less effective. A comprehensive characterization of the antibody response to blood stage malaria for both *P. falciparum* and *P. vivax* is required for the discovery of novel markers of both single and mixed clinical infections, as well as asymptomatic low density infections. Using recent developments in malaria genomic sequencing, proteomics, bioinformatics, high throughput cloning and proteome microarray fabrication technologies, we have constructed a blood stage proteome antigen array with a total of 4,000 recombinant proteins, which are the expression products of approximately 2,000 *P. falciparum* and 2,000 *P. vivax* blood stage ORFs. After recombinant cloning proteins were expressed using an E. coli based cell free expression system and printed directly on the nitrocellulose coated microarray slides without purification. Using this protein chip, sera from both symptomatic and asymptomatic children with *P. falciparum* and/or *P. vivax* infections from Papua New Guinea were screened. This approach will provide new insights into the correlation between antibody profiles and disease states that will lead to the characterization of serological correlates of active and past infection. These proteins are potential biomarkers that can be used for the development of diagnostic tools for the detection and characterization of co-infections, or for sero-surveillance markers.

1200

NO CORRELATION BETWEEN PARASITEMIA AND IGG ANTIBODY RESPONSE AGAINST *PLASMODIUM FALCIPARUM* GLUTAMATE-RICH PROTEIN (GLURP-R2) IN SERUM SAMPLES OF PATIENTS FROM IQUITOS, LORETO

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The highly antigenic *Plasmodium falciparum* GLURP-R2 protein is expressed in all stages of the parasite life cycle in human. It is considered as an important vaccine candidate antigen because its interaction with human IgG may play an important role in the development of clinical immunity. The aim of this study was to evaluate the IgG antibody response induced by GLURP R2 antigen in serum samples of patients infected with *P. falciparum* by indirect ELISA. Serum samples from 47 patients, between 9 and 63 years-old, mostly adults, infected with *P. falciparum*, were collected mainly in San Juan and Atalaya districts (province of Maynas), department of Loreto. All samples were positive by PCR and microscopy. Most patients from Atalaya community were asymptomatic, who showed low levels of parasitemia (from 24 to 7477 parasites/μL), while other communities showed higher levels of parasitemia (from 2162 to 61185 parasites/μL). Eight samples of people without any history of malaria disease were used as negative controls. Serum from patients infected with *P. vivax* was used to confirm the specificity of the assay. The cutoff value was calculated using the mean Optical density (OD) plus three standard deviations of negative control group. 87.23% (41/47) and 12.77% (6/47) were seropositive and seronegative to GLURP R2, respectively. The statistical significance of the correlation between parasitemia and IgG response level in both seropositive and seronegative groups was calculated with 95% of confidence ($p<0.05$). There was a weak inverse correlation between IgG response versus Log (parasites/μL) ($R^2=0.303$) for the seropositive group and a direct correlation for the seronegative group ($R^2=0.7806$). In addition, there was no correlation between the IgG response and parasites/μL neither with age or sex of the patient. In conclusion, the absence of significant correlations found shows that the immune response is influenced by other factors either intrinsic or extrinsic to the patient and that GLURP would not be a good vaccine candidate applicable to this region.

1201

PLASMODIUM FALCIPARUM DRUG RESISTANCE MOLECULAR MARKERS UNDER INTERMITTENT PREVENTIVE THERAPY WITH DIHYDROARTEMISININ-PIPERAQUINE (DP) VS. AMODIAQUINE-SULFADOXINE/PYRIMETHAMINE (AQ-SP) IN BURKINA FASO

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Single nucleotide polymorphisms (SNPs) in the *Plasmodium falciparum* pfcrt, pfmdr1, pfdhfr and pfdhps genes are associated with decreased response to aminoquinoline and antifolate antimalarials and have been shown to be selected by use of these drugs. The degree of selection by intermittent preventive therapy (IPT) regimens is unknown. We assessed the baseline prevalence and selection of common SNPs by IPTc in children in Bobo-Dioulasso, Burkina Faso. We studied 1500 children (aged 3-59 months) randomized to receive monthly dihydroartemisinin-piperaquine (DP) or amodiaquine-sulfadoxine/pyrimethamine (AQ/SP) for 3 months during the malaria transmission season in 2009. From random samples of 120 children for each arm of the study and for 120 of 250 untreated controls we evaluated the prevalence of key resistance-mediating SNPs. We then assessed the prevalence of the same SNPs in samples collected in November, 1 month after the third dose of IPTc. Before therapy malaria prevalence was 52.2% (188/360) based on microscopy and 66.67% (240/360) measured by PCR. Prevalences of SNPs were 68.5% (178/260) for Pfcrt 76T; 29.1% (75/258), 58.5% (151/258) and 7.70% (20/260) for Pfmdr1 86Y, 184F and 1246Y, respectively; 58.1% (151/260), 54.8% (143/261), and 55.0% (143/260) for Pfdhfr 51I, 59R and 108N, respectively; and 35.1% (91/259) and 56.8% (147/259) for Pfdhps 436S and 437G. After three monthly IPTc, AQ-SP selected significantly for mutant sequence pfcrt 76T, pfdhfr 59R, 108N and triple mutant 51I, 59R and 108N. DP did not select for known polymorphisms associated with aminoquinoline and antifolate resistance. Our result indicated that IPTc with AQ-SP selected for polymorphisms linked to resistance to AQ and SP probably because of increasing use of these drug. IPTc with DP do not select for known polymorphisms associated with drug resistance. DP may therefore be an excellent alternative for malaria prevention in children in Burkina. Nevertheless, further investigations are needed to confirm this absence of resistant parasite selection following IPTc with DP.

1202

IDENTIFICATION OF A KUPFFER CELL RECEPTOR FOR *PLASMODIUM* SPOROZOITE RECOGNITION

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After inoculation by the bite of an infected mosquito, the *Plasmodium* sporozoite enters the blood stream and infects the liver with unique specificity. To establish a productive hepatocyte infection sporozoites must find and traverse a Kupffer cell, a macrophage-like cell that lines the liver sinusoids. Using a phage display library we identified the P39 peptide that appears to mimic a sporozoite ligand for Kupffer cell recognition. Importantly either preincubation of rat Kupffer cells with P39 peptide or preincubation of *P. berghei* sporozoite with an anti-P39 antibody, inhibits sporozoite entrance into Kupffer cells. We determined that the P39 peptide binds specifically to a ~110 kDa Kupffer cell membrane protein and hypothesize that this protein acts as a sporozoite receptor for Kupffer cell traversal.

1203

CONTRASTING PATTERNS OF SELECTION ON THE ORTHOLOGOUS GENES ENCODING MEROZOITE SURFACE PROTEINS 4 (MSP-4) AND 5 (MSP-5) IN *PLASMODIUM* SPP.

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Merozoites are the invasive form of the malarial blood-stage life-cycle, exposing the merozoite surface proteins (MSPs) on their surface that are involved in initial attachment to the erythrocytes. Given the role of these proteins during the invasion of the red blood cell (RBC), several of them have been considered potential vaccine candidates. Among the MSPs, Merozoite surface protein 4 (MSP-4) and 5 (MSP-5) have received attention since these proteins share crucial structural features. They are glycosylphosphatidylinositol (GPI)-anchored integral membrane proteins with one epidermal growth factor-like domain (EGF) at the C-terminal. In addition, the genes encoding MSP-4 and MSP-5 are closely linked on the genome downstream from the gene encoding the highly conserved enzyme adenylosuccinate lyase (ASL). A single protein (MSP4/5) considered similar to both proteins has been identified in the three rodent malaria species; such a gene has lead to the hypothesis that MSP-4 and MSP-5 originated as a result of an early duplication event. In this study, we investigated the genetic diversity of orthologous genes encoding the MSP-4 and MSP-5 among *Plasmodium* species found in non-human primates that are closely related to *P. vivax*. We also evaluate the hypothesis that these genes are the result of an early duplication event during the evolution of *Plasmodium* in mammals. Overall, we found contrasting patterns of selection acting in genes encoding MSP-4 and MSP-5 in *P. vivax* and related species; MSP-5 orthologs are twice as polymorphic as MSP-4. In addition, we found that the polymorphism in MSP-4 in all *Plasmodium* species included in this study appears to be neutral. In contrast, we found evidence suggesting that MSP-5 in *P. vivax*, *P. cynomolgi* and *P. inui* is under positive selection. Our results reveal that exon I exhibits significant more non-synonymous than synonymous substitutions, confirming previous reports in *P. vivax*. This finding suggests that MSP-5 may be under selective pressure by the immune system across different species of primates including humans.

1204

EXTERNAL QUALITY ASSURANCE PROGRAM FOR *PLASMODIUM FALCIPARUM* RECRUDESCENCE-REINFECTION GENOTYPING IN ANTIMALARIAL DRUG EFFICACY STUDIES

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The authors present this on behalf of the Molecular Surveillance Network for Malaria Drug Resistance in the Greater Mekong Subregion.

The Molecular Surveillance Network is a collaborative effort aiming to strengthen regional and global malaria control and elimination programs by improving quality and comprehensiveness of surveillance for drug resistance and efficacy. The Molecular Surveillance Network partnership includes national malaria control programs of countries in the Greater Mekong Subregion (Cambodia, China, Lao PDR, Myanmar, Thailand, Vietnam) and supports molecular laboratories performing *msp1*, *msp2*, and *glurp* genotyping to distinguish recrudescence from reinfection (RvR) in therapeutic antimalarial drug efficacy studies. Because non-kit-based assays such as RvR testing are difficult to standardize, wide discrepancy can be observed in test results. Factors contributing to this variation include laboratory-laboratory variations in equipment, reagents, supplies and procedures, and the subjective nature of result interpretation, here size-scoring bands on agarose gels. Proficiency testing (PT), an important component of external quality assurance, assesses participants' ability to obtain true results for a set of samples. The Molecular Surveillance Network PT program is a voluntary, confidential testing scheme open to laboratories performing RvR testing on dried blood spot samples. PT panels consist of paired dried blood spots corresponding to pre-treatment (day 0) and post-treatment initiation (day of recurrent parasitemia) samples. Panels are incorporated into routine testing and results are sent to the PT program's organizers for feedback. The PT program was pilot-tested in four laboratories prior to a regional RvR training workshop. Elements of non-conformity included absence of control samples, failure to include gel photos for interpretation and incomplete labeling of results. A post-workshop PT round involving five laboratories resulted in notable improvements in standardization of procedures, use of controls and labeling of samples. Although PT is most powerful when used for quantitative tests with statistically significant numbers of participants, we show that a qualitative, small-scale pilot program for a non-kit-based molecular assay can result in discernable quality improvements.

1205

CHARACTERIZATION OF PFNPC1, A PUTATIVE LIPID TRANSPORTER IN *PLASMODIUM FALCIPARUM*

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During the blood stage of the *Plasmodium* life cycle, the malaria parasite replicates within the erythrocyte, giving rise to 8-32 daughter merozoites. This expansion necessitates large amounts of fatty acids to supply membranes to the newly formed daughter cells. In theory, these fatty acids can either be synthesized by the parasite or acquired from the host. However, recent reports have demonstrated that parasites lacking key enzymes in the fatty acid synthesis pathway have no defect in replication during the blood stage. This suggests that fatty acid acquisition pathways are essential for asexual blood stage development. Indeed, it has been demonstrated that *P. falciparum* requires exogenous sources of both palmitic and oleic acid during blood stage growth. We have initiated studies to define the mechanisms by which fatty acids are imported into intra-erythrocytic parasites. Our studies focus on the role of the *Plasmodium* Niemann-Pick C1 protein homologue, PfNPC1 and its potential role in lipid import. PfNPC1, like its mammalian homologue, NPC1, consists of a sterol sensing domain, a "patched" domain and three large loops. PfNPC1 is expressed during the ring and early trophozoite stage. Fluorescence microscopy of parasites expressing C-terminal GFP-tagged PfNPC1 reveal that this protein is localized to the parasitophorous vacuole, a location that would facilitate the import of host-derived fatty acids. Immuno-electron microscopy is being used to dissect the precise membrane on which this protein resides. Attempts to generate a PfNPC1 knock out have been unsuccessful, suggesting that the protein has an essential function during the blood stage. Ongoing studies aim to elucidate the function of this protein using a conditional knock-down system.

1206

ANALYSIS OF FIELD ISOLATES FROM A CHRONIC *PLASMODIUM FALCIPARUM* INFECTION SUGGESTS THAT VARIANT SURFACE ANTIGENS ARE NOT EXCLUSIVELY COMPOSED OF PFEMP1

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Antigenic variation of variant surface antigens (VSA) enables *Plasmodium falciparum* to establish chronic infections. *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1) are suggested to be the major cause of antigenic variation. PfEMP1 is encoded by the multicopy *var* gene family. We have shown that *var* gene transcription in the 3D7 genome strain and in field isolates is biased towards central UpsC *var* genes (Enderes et al. submitted). This raises the question how *P. falciparum* escapes the immune response if it constantly expresses an individual *var* locus. Here we employ parasites and sera from an asymptomatically infected individual to investigate the determinants of antigenic variation. We used shotgun cloning to characterize the *var* gene repertoire at different time points of the infection. Fluorescence activated cell sorting (FACS) was employed to characterize the humoral immune response. To determine individual targets of the antibody response we generated PfEMP1 knock-down parasites in field isolates as well as in NF54 laboratory clones. In these parasites drug pressure removes PfEMP1 from the erythrocyte surface. CD36 receptor binding was used to select for PfEMP1 expression in all parasite lines.The *var* gene repertoire was identical at all time points of the infection, underscoring the parasites ability to evade the human immune response. FACS with sera of the infected individual displayed a strong signal in culture adapted field parasites but not in NF54. However, selection for CD36 binding induced a strong FACS signal in NF54 parasites suggesting crossreactivity with VSA. Application of drug pressure to transgenic NF54 parasites removed this signal, indicating that these antibodies are directed against PfEMP1. Surprisingly, drug pressure had no effect on the FACS signal of transgenic field isolates. This suggests that a large part of the epitopes on these field isolates are not PfEMP1. Taken together our data suggest that antigenic variation is not exclusively mediated by PfEMP1. Transgenic field isolates may provide new insights into the mechanisms mediating immunity to *P. falciparum* malaria.

1207

POPULATION GENETIC INFERENCES OF *PLASMODIUM FALCIPARUM* BASED ON FULLY SEQUENCED GENOMES FROM SENEGAL

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Malaria is a deadly disease that causes nearly one million deaths each year. Understanding the demographic history of the malaria parasite *Plasmodium falciparum* and the genetic basis of its adaptations to antimalarial treatments and the human immune system is important for developing methods to control and eradicate malaria. To study the demographic history and identify genes under selection more efficiently, we sequenced the complete genomes of 25 cultured *P. falciparum* isolates from three cities in Senegal. Based on genetic diversity of the genome sequences, we estimate the long-term effective population size to be approximately 100,000 and show that there is no significant population structure within Senegal. We also estimate a major population expansion of the parasite population approximately 550,000-770,000 years ago. By using the results on demographic history as a null model, the sequences also reveal candidate genes under selection, including pfcrt and dhfr. Moreover, the rates of decay of linkage disequilibrium are fast, indicating the potential of fine-scale genetic mapping in *P. falciparum*.

1208

VARIATION WITHIN THE TOLL-LIKE RECEPTOR-9 (TLR-9) GENE PROMOTER (-1486T/C) IS ASSOCIATED WITH INCREASED SUSCEPTIBILITY TO PEDIATRIC SEVERE MALARIAL ANEMIA AND FUNCTIONAL CHANGES IN CIRCULATING IFN-Γ

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Plasmodium falciparum malaria is one of the leading causes of infectious disease burden in the world. In holoendemic *P. falciparum* transmission areas, such as western Kenya, severe malarial anemia [SMA, hemoglobin (Hb)<5.0g/dL] results in high rates of pediatric morbidity and mortality. Since Toll-like receptors (TLRs) affect innate and adaptive immune responses, the functional roles of polymorphic variants within the TLR-9 gene in conditioning susceptibility to SMA were investigated. Specifically, the relationship between the TLR-9 variant (-1486T/C, rs187084) and susceptibility to SMA (Hb<5.0 g/dL, any density parasitemia) was investigated in children (n=468) with *falciparum* malaria from a *P. falciparum* holoendemic transmission region in western Kenya. Hematological and parasitological profiles were determined in all study participants. TLR-9 -1486T/C genotypes were determined using TaqMan 5' allele discrimination assay. Circulating interferon (IFN)-γ levels were measured using Biosource™ hu-multiplex inflammatory profile. Frequencies of the -1486TT, TC and CC were 54.4%, 30.7%, and 14.9%, respectively. Multivariate logistic regression analyses controlling for potential confounders demonstrated that homozygous C individuals (OR; 1.68, 95% CI, 1.02-2.77; *P*=0.041) were associated with increased susceptibility to SMA relative to TT individuals. In addition, carriers of the CC genotype had significantly higher circulating IFN-γ levels relative to TT (*P*=0.046). Findings presented here demonstrate that variation in TLR-9 at -1486 is associated with increased susceptibility to SMA and functional changes in circulating IFN-γ levels.

1209

HIGH-DENSITY GENOTYPE ANALYSIS OF A DEEP AFRICAN SAMPLE OF *PLASMODIUM FALCIPARUM*

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We have used a high-density SNP genotyping array (querying 17,000 sites) to examine population structure and differential evidence of natural selection in a set of 93 *P. falciparum* samples from Senegal and the Gambia. After correcting for a small but measurable effect from sample preparation (culture-adapted vs. non-culture-adapted parasites), we find no statistically significant difference in allele frequencies between the two countries, despite different histories of drug use. This suggests that regional data collection should be adequate for genome-wide association studies on this scale. We also examined linkage disequilibrium and find considerable variation across the genome, only some of which can be explained by previously identified selective sweeps. We are continuing our

analysis, including long-range haplotype tests for positive selection, to determine whether these regions represent additional sweeps or areas of reduced recombination.

1210

INVASION OF *PLASMODIUM FALCIPARUM* FIELD ISOLATES FROM SOUTH AMERICA: PHENOTYPIC AND GENOTYPIC ANALYSES

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Invasion of RBCs by *Plasmodium falciparum* involves multiple pathways including those utilizing ligands of the Erythrocyte-Binding Ligand (EBL) and the Reticulocyte-Binding protein homolog (PfRh) families. The invasion of 20 South American (SA) field isolates from Colombia (Antioquia), Peru (Iquitos) and Brazil (Pará) was studied. Seven different invasion profiles were found, one of which is independent of neuraminidase (N), trypsin (T) and chymotrypsin (C) sensitive receptors (NrTrCr), and which was not previously reported. This pathway was used predominantly by Colombian and Peruvian field isolates with varying levels of resistance to the three enzyme treatments (58-93%). Regrettably, majority of other invasion studies did not examine the chymotrypsin treated RBCs for their invasion profile classification. However, even when only the use of the NrTr invasion pathway was compared between the SA isolates and those studied previously, it appeared that 46% of the SA isolates use this pathway in contrast to <5% by African and Brazilian (Mato Grosso) isolates. The use of chymotrypsin treated RBCs allowed us to evaluate the involvement of GPB, and the unknown receptors of EBA-181, PfRh2b and PfRh4 in the alternative invasion pathways of the SA isolates, and which appeared to be more predominant in the Brazilian isolates (5/7). The specific dependence on GPB for invasion was further estimated by using GPB-negative RBCs and the differential use of this receptor vs. the other chymotrypsin sensitive receptors will be presented. Two distinct dominant clusters of invasion profiles were found in SA field isolates: NrTsCs in Brazil, and NrTrCr in Colombia and Peru, both of which are different of those present in Africa, and in part more similar to the Indian field isolates. When the polymorphic variants of the PfRh and EBA-181 and EBL-1 ligands were compared to lab strains and the Mato Grosso field isolates, we found some novel variants in the Peruvian and Colombian field isolates. The association between ligand polymorphisms and the differing invasion pathways used by the SA parasites will be discussed.

1211

USING CF11 CELLULOSE COLUMNS TO QUICKLY, INEXPENSIVELY AND EFFECTIVELY REMOVE HUMAN DNA FROM *PLASMODIUM FALCIPARUM*-INFECTED WHOLE BLOOD SAMPLES

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Next-generation Illumina® sequencing of *Plasmodium* genomes requires depletion of human DNA from parasitized whole blood samples prior to extraction, storage and shipping of DNA to sequencing facilities. The most effective method currently in use is a two-step procedure to deplete leukocytes: centrifugation using density gradient media followed by gravity filtration through expensive, commercially-available columns. This method is not easily implemented in studies collecting hundreds of samples, processing samples for multiple laboratory analyses simultaneously, or lacking capacity for refrigerated centrifugation. Inexpensive syringes hand-packed with CF11 cellulose powder were recently shown to improve ex vivo cultivation of *Plasmodium vivax* obtained from parasitized whole blood samples, as reported previously. We have adapted this procedure to isolate *P. falciparum* DNA from *in vitro* cultured parasites and parasitized whole blood samples obtained ex vivo from Cambodian patients with malaria. Using this method to process blood samples of at least 2 mL and containing at least 10,000 parasites per microliter, we reliably produced 500 nanograms of parasite DNA with less than 30% human DNA contamination. This sample profile is comparable to that obtained by the two-step method and falls well within the current quality control requirements for Illumina® sequencing. In addition, we have validated a centrifuge-free version of the CF11 filtration method to isolate *P. falciparum* DNA at remote and minimally-equipped sites in malaria-endemic areas.

1212

GENETIC DIVERSITY IN *PLASMODIUM FALCIPARUM* MSP GENE FOR 7 DAYS POST-TREATMENT CHARACTERIZE TREATMENT FAILURES IN AN ARTESUNATE MONO-THERAPY TRIAL IN WESTERN CAMBODIA

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Diversity in the *Plasmodium falciparum* genes encoding merozoite surface proteins (*msp) msp1 and msp2*, and *glurp*, has implications for the epidemiology of malaria and the efficacy of malaria drugs. The WHO standard for determining whether a treatment failure is a recrudescence or a new infection uses matched samples from baseline (D_0) and day of failure (D_f). However, in a region of emerging drug resistance, the proportion of parasite at D_0 susceptible to drug may be large and mask a

minute proportion of parasites that are drug resistant. We hypothesized that a small population of resistant parasites may escape drug therapy undetected and reemerge later at the time of treatment failure. In this scenario, a specimen from Day 3 of treatment would better represent parasites that are more resistant to drug and that may actually lead to treatment failure. In a randomized study conducted in an area of emerging artemisinin resistance in western Cambodia during 2008-2009 the efficacy of 7-day courses of artesunate monotherapy for the treatment of uncomplicated *falciparum* malaria were assessed. Samples for nested PCR were collected pre-treatment, on days 2, 3, 4, 5 and 6 of treatment, and on the day of failure (D_f). Patterns of allelic diversity of *msp* and *glurp* were used to distinguish between recrudescence and reinfection by nested PCR. 143 patients were enrolled of who 10 were classified as late treatment failures, 2 as reinfection and 8 recrudescent. A high proportion of isolates from recrudescent subjects showed multiple *msp* allelic types on D_0 and D_f, consistent with a heterogeneous *falciparum* infection. For some subjects, in comparison with D_0, some alleles disappeared by day 3, and re-appeared on D_f. In conclusion, for assessing re-infection and recrudescence in malaria treatment trials, the inclusion of *msp* allelic analysis on post-treatment days 2 through 7, especially day 3, may be a useful addition to the current WHO standard of D_0 and D_f for characterizing allelic distribution.

1213

APPLICATION OF NEXT GENERATION SEQUENCING TO SEARCH FOR ALLELE-SPECIFIC IMMUNITY IN AN RTS,S CLINICAL TRIAL IN MOZAMBIQUE?

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Most candidate malaria vaccines, including the RTS,S vaccine that is now undergoing Phase III trials in Africa, are subunit protein vaccines based on highly polymorphic loci. The goal of this study is to understand the impact of vaccination on the diversity of the parasite population in individual patients. The underlying hypothesis is that successful vaccination is allele-specific and we have tested this hypothesis by comparing parasite populations in RTS,S vaccinated versus control vaccinated individuals. Specifically, we have analyzed the association between parasite diversity at the CSP locus, the antigen target of the RTS,S vaccine, and vaccination in patients who contracted infection during a Phase IIb RTS,S trial conducted in Mozambique. One of the key technical developments necessary to conduct this work is the ability to both detect and quantify the parasite populations within a single patient. To achieve this goal, we developed a new genotyping approach that utilizes next generation sequencing to recover CSP haplotypes in both mixed and single clone infections. Haplotypes capture complex interactions between polymorphisms and are directly associated with protein production and parasite fitness. The sequenced reads were filtered to remove error-prone and misaligned reads and then clustered by similarity into haplotypes. Using model-based filters, we identified and removed SNP errors and chimeras that arise during sample preparation and sequencing and further improved the accuracy of haplotype identification. This approach successfully recovers rare haplotypes (at a frequency of 1%) and yields a sensitive measurement of multiplicity of infection (MOI). The approach has been validated in *in vitro* mixtures of parasites. We assessed whether the parasite populations were different between RTS,S-vaccinated and comparator-vaccinated subjects with respect to specific haplotypes and MOI. Additionally, we re-visited the Enosse et al (2006) analysis to assess whether the increased efficacy of the RTS,S vaccine against severe disease could be attributed to a decrease in MOI. This analysis strategy should prove useful for evaluating allele-specific efficacy in the context of other malaria vaccine trials.

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TRANSMISSION BLOCKING ACTIVITY OF ANTIBODIES RAISED AGAINST A PFS25- BASED VACCINE DERIVED FROM NF54 SEQUENCE AGAINST FIELD ISOLATES FROM THAILAND

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Eradication of malaria is possible by the interruption of local mosquito borne malaria transmission and is the end goal in the fight against the disease. Transmission-blocking vaccines (TBVs) that target the sexual stage react with the ookinete surface proteins of malaria parasites within the mosquito midgut, which will contribute to elimination of the disease by blocking the parasite transmission. Pfs25 is a lead TBV candidate, and a Pfs25-based vaccine, Pfs25/ISA51 has been tested in a Phase 1 trial. The vaccine was produced using the Pfs25 sequence from NF54 isolate. Since only a limited sequence polymorphism was reported for this gene, we hypothesized that anti-Pfs25 antibodies induced by this vaccine will have transmission blocking activity against most, if not all, field isolates. To test this hypothesis, we evaluated transmission blocking activities of anti-Pfs25 plasma from the Pfs25/ISA51 trial against parasites in blood of *Plasmodium falciparum* infected patients in Thailand. Normal human Plasma was used as controls. The blood was drawn from each patient and was first tested for transmission blocking activity by membrane feeding assay in triplicates. In parallel, blood samples from these patients were spotted on filter papers for sequencing of Pfs25 genes and for genotyping analysis to determine the independent origin of the parasites. Despite the different genetic background, the Pfs25 sequences from these parasites are identical. The transmission blocking activities of the plasma against these parasites in different blood samples are comparable. Percent reduction in oocyst count in membrane feed assay, when immunized plasma compared with normal plasma is highly significant ($P<0.0001$).

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CLINICAL TRIAL OF THE SANARIA® PFSPZ VACCINE VIA THE INTRAVENOUS ROUTE - RATIONALE, PLANS AND PROGRESS

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Immunization by the bites of mosquitoes infected with radiation-attenuated *Plasmodium falciparum* sporozoites remains the most effective method for inducing sustained, high-level protective immunity in humans not treated with anti-malarials. To advance this concept, the *Plasmodium falciparum* Sporozoite (PfSPZ) Vaccine, comprising metabolically-active, non-replicating, purified, aseptic, cryopreserved parasites, has been developed. In the first human trial of the PfSPZ Vaccine, immunization of healthy malaria-naïve volunteers by the subcutaneous (SC) and intradermal (ID) routes was safe and well-tolerated. However, both immunogenicity and protective efficacy were suboptimal. Recent experiments in mice, rabbits, and especially non-human primates (NHPs) demonstrate that the PfSPZ Vaccine is highly potent and that immunogenicity and protective efficacy are far superior when administered intravenously (IV) as compared to SC or ID. In NHP, high levels of SPZ specific CD8+/IFN-g producing cells

were detected in the livers several months after a series of IV but not SC immunizations. *In vitro* data demonstrate that irradiated, aseptic, purified, cryopreserved PfSPZ can invade NHP hepatocytes, providing a potential explanation for such potent responses. Furthermore, administration of labeled SPZ in mice confirm substantially greater distribution of the vaccine to the liver after IV than after SC administration. Together, these animal studies provided the rationale for assessing IV administration of the PfSPZ Vaccine in a Phase 1 clinical trial with experimental challenge. This dose escalation trial is designed to maximize volunteer safety and to provide 1) a clinical proof of principle, 2) a foundation for a clinical development plan leading to licensure of IV-administered vaccine for targeted market segments and 3) a benchmark for development of a non-IV parenteral mode of administration.

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VACCINE CANDIDATE IDENTIFICATION FOR PEDIATRIC *FALCIPARUM* MALARIA

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Plasmodium falciparum remains a leading cause of morbidity and mortality in developing countries and vaccines for this parasite are urgently needed. Human residents of endemic areas develop protective immunity that limits parasitemia and disease, and naturally acquired human immunity provides an attractive model for novel vaccine antigen identification. As part of the MOMS project, 785 Tanzanian children living in an area of intense malaria transmission were enrolled at birth, and intensively monitored for parasitemia and clinical illness for up to 3 yrs, with an average of 47-blood smears/child. We identified resistant (n=10) and susceptible (n=10) children based on the results of monthly blood smears obtained from the age of 2 to 3 yrs with matching for potential confounders. Using a differential library screening approach, we identified parasite genes that encode proteins uniquely recognized by plasma pooled from resistant, but not susceptible children. We characterized these candidates with western blot and immunolocalization assays and validated them with independent selections of plasma and with growth inhibition assays. We screened 750,000 clones and identified 3 clones uniquely recognized by resistant but not susceptible plasma. These encoded MSP-7, and hypothetical proteins on chromosomes 10 and 11. We expressed and purified clone 10 and generated anti-sera which, in accordance with *in silico* predictions, recognized a 244 kDa antigen in *P. falciparum* infected, but not uninfected RBCs. In growth inhibition assays, anti-clone 2 anti-sera inhibited parasite growth by 48-63% in several parasite strains. In an ELISA assay using independent selections of resistant (n=11) and susceptible (n=14) plasmas, resistant individuals had 4 fold higher antibody levels to clone 2 proteins compared to susceptible individuals. In conclusion, our differential screening approach identified several novel vaccine candidates and we are currently evaluating the relationship between antibody levels to clone 2 and resistance to infection and disease in the entire birth cohort.

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A NEW MALARIA EXPERIMENTAL CHALLENGE SYSTEM: INFECTION OF VOLUNTEERS BY THE BITES OF ASEPTIC *ANOPHELES STEPHENSI* MOSQUITOES INFECTED WITH *PLASMODIUM FALCIPARUM* (NF54) SPOROZOITES

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Experimental malaria sporozoite challenge is an essential component of the vaccine development plan for malaria vaccine candidates targeting pre-erythrocytic stages of the parasite. The current challenge model requires the bites of five infected mosquitoes raised in traditional insectaries to consistently induce malaria. We previously reported on an improved malaria challenge system using the bites of one, three or five aseptically-raised mosquitoes in compliance with cGMP and demonstrated that the aseptic model is safe, associated with a precise prepatent period, and transmitted malaria to all six participants bitten by three *Anopheles stephensi* mosquitoes. As a follow-up study, we evaluated the aseptic model using the bites of three mosquitoes in nineteen additional malaria-naïve adults. In total, twenty-five adults aged 18-40 years (mean= 30 years) were bitten by three A. stephensi mosquitoes infected with the NF54 strain of chloroquine-sensitive *P. falciparum*. The geometric mean sporozoite count detected in challenge mosquitoes was 36.1 x 10^3 (range 6.0-71.1 x 10^3). All twenty-five participants developed microscopy-confirmed peripheral parasitemia, seventeen (68%) on Day 11 post-challenge. The mean prepatent period was 10.9 days (range 9-14 days). The mean parasitemia at diagnosis was 10.8 parasites/µL (range 2-44). Polymerase chain reaction detected malaria in all participants prior to microscopy (mean 3.4 days, range 2-5). No serious adverse events were encountered. The most common solicited events included headache, chills, myalgia, and fever. The aseptic, cGMP-compliant challenge model using three infected mosquitoes transmitted malaria to 100% of participants. The cGMP system provides reliable infection and improves the challenge model by establishing a foundation for assessing the infectivity of sporozoites from aseptic mosquitoes after they have been extracted, purified, vialed, cryopreserved, thawed, and administered by needle and syringe.

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PHASE 1 STUDY OF THE SAFETY AND IMMUNOGENICITY OF BSAM-2/ALHYDROGEL®+CPG 7909, AN ASEXUAL BLOOD STAGE VACCINE FOR *PLASMODIUM FALCIPARUM* MALARIA IN ADULTS IN MALI

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A single blind, randomized, controlled Phase 1 clinical trial is being conducted to assess the safety and immunogenicity in malaria exposed adults of the *Plasmodium falciparum* blood stage vaccine BSAM-2, containing a four recombinant protein mixture of AMA1 (AMA1-FVO+AMA1-3D7) and $MSP1_{42}$ ($MSP1_{42}$-FVO+$MSP1_{42}$-3D7) /Alhydrogel® with the novel adjuvant CPG 7909. Participants are healthy adults 18-45 years old living in the village of Bancoumana, Mali. Thirty participants

have received 3 doses (Days 0, 56, and 120) of either BSAM-2 or Euvax B/Hepatitis B vaccine and followed actively for 8 months after the last vaccination and passively for an additional of about 2 months. Enrollment and first vaccinations occurred in March and April of 2010. Vaccinations were well tolerated, with related adverse events being mostly mild or moderate injection site reactions. Antibody responses for AMA1 and $MSP1_{42}$ were higher in the group receiving BSAM-2 for all time points after the first vaccination and the differences were statistically significant ($p<0.05$). There was no significant increase in antibody levels 14 days after the third vaccination compared to 14 days after the second vaccination. The incidence rate of clinical malaria was similar between the vaccination and comparator groups. Despite the favorable safety profile and good immunogenicity, no further clinical development of BSAM2/Alhydrogel®+CPG 7909 is currently anticipated.

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OPTIMIZATION OF A MOUSE CHALLENGE MODEL TO EVALUATE THE EFFICACY OF *PLASMODIUM FALCIPARUM* CIRCUMSPOROZOITE PROTEIN BASED MALARIA VACCINES

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Future improvements in the protective efficacy of Circumsporozoite protein based vaccines will depend on preclinical data comparing delivery platforms and mixed antigen combinations. Transgenic rodent parasites where the native CSP has been replaced with a functional PfCSP gene will be important tools for down-selecting vaccine candidates. We obtained a transgenic rodent parasite line in which the full-length PfCSP gene was inserted into the *Plasmodium berghei* genome, as reported previously. The parasite was adapted to grow at the WRAIR entomology laboratory using serial passages of sporozoite induced and blood induced infections. We confirmed the transgenic nature of the parasite by IFA with species-specific monoclonal antibodies to CS. We observed normal oocyst development, but significantly reduced salivary gland sporozoite burden in mosquitoes. The minimum infective dose of the sporozoites was established and a series of challenge experiments were conducted comparing several PfCSP based protein vaccine candidates. Protection was defined as complete absence of blood stage parasites on day 15 post challenge. Using a challenge model to down-select vaccine candidates can have important implications for improving CSP based vaccine candidates.

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A NOVEL TRICK TO CONTROL MALARIA: MANIPULATING THE MOSQUITO INNATE IMMUNE RESPONSE AGAINST *PLASMODIUM* PARASITES TO BLOCK TRANSMISSION

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Malaria is caused by the protozoan parasite *Plasmodium* which is transmitted by the female *Anopheles* mosquito. The parasite must complete its sexual development in the mosquito before it can be transmitted to the human host. The innate immune response of the mosquito considerably hinders the development of the parasite but this is often not sufficient to clear the infection. In natural infection of the mosquito by *Plasmodium*, there has to be a fine balance between the immune response against the parasite and immune pathology which is reportedly detrimental to the health of the mosquito. We have tried to tip this balance in favour of the mosquito's immune system, which will hinder parasite development and reduce malaria transmission. We have used a viral vectored vaccine platform to express candidate antigens, which are components of the mosquito's innate signalling pathways. Mice have been vaccinated and serum used to measure transmission-blocking activity of antibodies generated by immunization using standardized readouts of *in vivo* efficacy and effect on mosquito survival. This novel strategy could be a revolutionary breakthrough as it would not only work against potentially all four malaria species that infect humans, but likely also against some other mosquito-transmitted diseases and could have a major impact in decreasing the burden of vector-borne diseases. We have also used this vaccine platform to screen several leading parasite and mosquito based malaria transmission blocking vaccine candidates The significance of this work is to provide the first and much needed head-to-head assessment of the *in vivo* efficacy of the known leading TBV candidate antigens, as well as look for novel antigens aimed at de-regulating the mosquito's innate immune system in favour of transmission-blocking activity.

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ANTENATAL MALARIA AND HELMINTH INFECTIONS ARE ASSOCIATED WITH IMPAIRED VACCINE EFFICACY IN KENYAN INFANTS

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African pregnant women are often chronically infected with parasites whose soluble products can cross the placenta and prime or induce immunomodulatory responses in the fetus that can persist into infancy and could affect infant immune responses to childhood vaccines. To test this hypothesis we examined the effect of malaria, schistosomiasis, and intestinal parasites in pregnant Kenyan women (n=545) on the development of IgG antibody responses to tetanus, diphtheria, hepatitis B virus, Haemophilus influenzae type B (Hib), and poliovirus in their offspring following vaccination at 6,12,18,24,30 and 36 months of age. Overall 64.2% of the pregnant women were infected with helminths: 46% and 18% with single and multiple infections respectively. 29%, 20%, 15% and 10% were infected with schistosomiasis, hookworm, or malaria respectively. Children of mothers infected with malaria had lower diphtheria titers at 6, 12 and 18 months of age as compared to children of uninfected mothers ($P<0.01$-0.0009 at each time point estimated by generalized estimating equations). Similarly, offspring of schistosomiasis-infected versus uninfected women had lower diphtheria titers at 12 and 24 months of age ($P<0.01$). In contrast, offspring of schistosomiasis-infected compared to uninfected women had higher polio titers at 12, 18 and 24 months of age ($P<0.01$ at each time point). Children of mothers infected with 2 or more infections had significantly lower Hib-IgG levels at 12 months of age and higher polio-IgG levels at 18 months of age compared to children of mothers with single infection ($P<0.01$). There was no significant difference in antibody levels to any childhood vaccines in children of mothers infected with hookworm, Trichuris, or other intestinal helminths as compared to children of uninfected mothers. Thus, malaria and chronic helminth infections during pregnancy alters responses antibody responses to childhood vaccines and highlight the importance of national programs to eradicate malaria and helminth infections in pregnant women.

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CRY1AC PROTOXIN COADMINISTERED WITH *PLASMODIUM* ANTIGEN SYNERGIZES CATALASE ACTIVITY AND NO LEVELS ON CBA/CA MICE INFECTED WITH *P. BERGHEI* ANKA

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We have shown that Cry1Ac induces protection against *Plasmodium chabaudi* AS and *P. berghei* ANKA infection. In this work, we analyzed whether the coadministration of Cry1Ac protoxin with *P. berghei* ANKA antigen (Ag) potentiates this protection and if oxidative stress is associated to parasite elimination. Groups of CBA/Ca mice were weekly treated with: PBS, protoxin Cry1Ac, Ag plus PBS or Ag plus Cry1Ac (Ag+Cry) during 5 weeks, one day after the last injection, mice were infected with *P. berghei* ANKA. Parasitaemia, body weight and survival were recorded daily. In addition, on day 9 post infection splenic mRNA was isolated retrotranscribed and analysed for IFN-γ using qPCR, nitric oxide serum levels and catalase activity also were studied. Mice treated with Ag increased survival for 5 days while mice injected with Ag+Cry survived 8 days more compared to mice treated with PBS (control group), both groups of mice treated with Ag developed lower parasitaemias and lower spleen index compared to control group, furthermore, IFN-γ mRNA expression was upregulated, which implies that with lower cell proliferation the better parasite elimination was attained. Mice treated with Ag+Cry developed significantly higher levels of NO and catalase specific activity in the spleen compared to control group, all thse results suggest that Cry1Ac protoxin could be a potential adjuvant for a malaria vaccine.

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NOVEL APPROACH FOR THE IDENTIFICATION OF NATURAL IMMUNE BOOSTING TRANSMISSION-BLOCKING VACCINE AGAINST *PLASMODIUM FALCIPARUM*

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Antibodies recognizing the surface of *Plasmodium falciparum* zygotes and ookinetes are thought to be ideal for the immunological interruption of malaria parasite transmission from vertebrate host to mosquito.After primary vaccination, antibody responses to such antigens would be boosted during infection. Such an approach would have a advantage over current lead TBV candidates such as Pfs25 that do not naturally induce immune responses in humans because of very low or lack expression in the human host and/or low antigenicity. Here we propose that the identification of antigens shared between gametocytes,sporozoite and zygotes/ookinetes is a new approach to the development of transmission-blocking vaccines (TBV). We hypothesized that highly expressed ookinete surface proteins of *P. falciparum* that are also expressed by asexuals, gametocytes or sporozoites would boost transmission-blocking antibodies during natural malaria infection. To test this hypothesis, we used existing ookinete proteome data and a *P. falciparum* protein microarray to identify antigens that might boost transmission-blocking activity by being shared among blood and mosquito midgut stages. 110 African patient sera recognized 79 predicted ookinete surface proteins of *P. falciparum* (*P. gallinaceum* ookinete orthologs) on the protein microarray; several ookinete surface proteins were found also to be expressed in gametocyte, sporozoite or asexual blood stage parasites. The hypothetical PF11_0055 gene product contains a predicted thioredoxin-like domain, is highly conserved (79%) in *P. berghei*, is expressed in all stages, and was immunogenic. Vaccination of mice with recombinant E. coli-produced PF11_0055, followed by *P. falciparum* gametocyte lysate, boosted anti-PF11_0055 antibody titer compared to gametocyte lysate alone used as vaccine. In standard membrane feeding assays, antibodies to PF11_0055 antibodies significantly reduced oocyst numbers and infected mosquito prevalence. Another protein, PfCelTOS, a known sporozoite- and ookinete-expressed protein, was found to be abundant in ookinetes and highly immunogenic (spec count: ookinete 84, sporozoite 58; geometric mean titer 6796); polyclonal mice sera effectively blocked oocyst development in *P. falciparum*. This new approach to transmission-blocking vaccine candidate discovery based on systems biology antigen discovery is a promising new direction in malaria vaccinology.

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IMMUNODAMPENING TO OVERCOME DIVERSITY IN THE MALARIAL VACCINE CANDIDATE APICAL MEMBRANE ANTIGEN 1

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Apical membrane antigen 1 (AMA1) is a leading candidate for inclusion in a malaria vaccine however the polymorphic nature of this protein may limit its efficacy. Within AMA1, the highly variant loop Id has been identified as a dominant target of strain-specific, inhibitory antibodies. In this study we aimed to circumvent AMA1 diversity by dampening the immune response to loop Id and enhancing the response to more conserved epitopes. To achieve this, five polymorphic residues in loop Id were mutated to alanine, glycine or serine and initially the corresponding antigens were displayed on the surface of bacteriophage to assess their ability to fold correctly. Reactivity with conformation-sensitive antibodies indicated that glycine substitution compromised formation of the correct disulphide-bonded structure and the glycine mutants were therefore not produced as purified recombinant proteins. Since phage-based assays indicated that the alanine and serine mutants were correctly folded, these variants were expressed in *E. coli*, refolded *in vitro* and used to immunize rabbits. Serological analyses indicated that immunization with a single mutated form of AMA1 was sufficient to increase the cross-reactive immune response. Furthermore, combining engineered forms of AMA1 derived from two different alleles was more effective at broadening the immune response than combining the two corresponding wild type antigens. This suggests that inclusion of a mutated form of AMA1 in a malaria vaccine may reduce the number of variants required to induce a sufficiently broad immune response. We are currently expanding this study to determine which combination of wild type and/or mutant AMA1 offers the most promise for protection from diverse *Plasmodium falciparum* genotypes.

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LANDSCAPE OF RESPONSIBILITY: EVOLVING OF ROLES AND RESPONSIBILITIES FOR COMMUNITIES AND INSTITUTIONS IN A LARVAL CONTROL PROGRAM FOR MALARIA PREVENTION IN URBAN DAR ES SALAAM, TANZANIA

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In the first half of the 20th century, targeting the larvae of mosquitoes was regarded as a practical means to reduce malaria in cities. However, because it is labour intensive and demands considerable manpower, entomological expertise as well as institutional oversight, this approach fell out of favour for decades and is only recently being reconsidered.

This analysis describes a community-based programme for larval control of malaria vector mosquitoes in urban Dar es Salaam, Tanzania, as an example of how scientific research and public health governance can be mutually configured in a contemporary African city. Initiated by the Dar es Salaam City Council, the Urban Malaria Control Program (UMCP) was designed to investigate the effectiveness of community-based systems for applying microbial larvicides, to aquatic breeding habitats in reducing the prevalence of malaria. The UMCP aims to demonstrate the operational feasibility of integrating larval control into routine municipal services, relying exclusively for its implementation on community-owned resource personnel (CORPs). The UMCP was therefore, designed to transform Dar es Salaam into both a venue of local management and a site of knowledge production. Drawing on ethnographic and historical resources, we consider the socio-technical practices these parallel transformations entail. In particular, we are concerned with how 'participation in' and 'responsibility for' larval control is inter-articulated through scientific protocols, development practices, and the specific political history of Tanzania. Through an analysis of the activities of the CORPs, we suggest that public health governance should be understood within a series of partial and spatially-bound relationships: between residents, local government from neighbourhood to city level research institutions and the reproduction traits of specific mosquito species. We conclude that to enable scaling up of a community-based intervention to a sustainably effective programme at city or national level requires, first, attention to the political history of those relationships and, second, an understanding of how responsibility for malaria control and public health more broadly, is best distributed within the simultaneous contexts of a scientific evaluation and a government-led programme.

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PHYSICAL DURABILITY OF TWO TYPES OF LONG-LASTING INSECTICIDAL NETS (LLINS) AFTER TWO YEARS OF USE, MOZAMBIQUE 2008-2010

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Understanding the physical durability (PD) of long-lasting insecticidal nets (LLINs) is critical to guide malaria programs on the frequency of LLIN replacement. We conducted a prospective evaluation of LLIN PD after a distribution campaign in October 2008 in Nampula Province, Mozambique.During the LLIN campaign we tagged 1000 LLINs of two types (polyethylene [PT] and polyester [PS]) at six distribution sites (6000 LLINs tagged). The tagged LLINs were geo-located during a house-to-house survey one month after the campaign and a random sample of households (HHs) was selected. One and two years after the campaign, the selected HHs were surveyed and all tagged LLINs were collected. LLINs were stretched over a frame against a black background and all holes were quantified. The difference in total number of holes by LLIN type and year was analyzed by unadjusted chi-square and the median number of holes and inter-quartile range (IQR) by hole size was analyzed using Wilcoxon rank sum test. One year after distribution 164 out of 210 HHs were interviewed and 148 LLINs were recovered and assessed; 50 of 51 (98%) PT and 73 of 97 (75%) PS had at least one hole ($p<0.0004$). Two years after distribution, 197 out of 240 HHs were interviewed and 163 LLINs were recovered; 58 of 59 (98%) PT and 97 of 104 (93%) PS had at least one hole ($p = 0.15$). The median number and IQR of holes after one and two years of use, respectively, was 18 (9, 33) and 53 (28, 98) for PT and 4 (1, 12) and 15 (5, 45) for PS. For both years, PT had a statistically significant higher number of holes of all sizes compared to PS ($p < 0.0001$). We found significant proportions of LLINs are damaged already by year one, more so for PT than PS. How this damage to LLINs translates into loss of protection against malaria transmission is not yet known. Additional studies are needed to measure the impact of the number and size of holes and physical integrity of the LLINs on malaria transmission to define LLIN failure.

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FREE NET DISTRIBUTION: WILL A HANG-UP CAMPAIGN MAKE AN IMPACT ON USE?

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Insecticide treated nets (ITNs) are highly effective in reducing malaria morbidity and mortality when used appropriately and consistently. The Angolan Ministry of Health (MoH) recently revised its National Strategic Plan for 2011-2015 to expand ITN coverage beyond pregnant women and children under five to universal coverage. An effective method to reach and maintain high net coverage is free distribution campaigns. Post-distribution hang-up campaigns to assist in and ensure utilization of ITNs have been implemented in several sub-Saharan countries. Survey results to evaluate the effectiveness of these campaigns indicate higher use of ITNs. In Angola, the first major free net distribution campaign targeting universal coverage is currently underway (April-August 2011). Africare is implementing the campaign in thirty-two communities in four municipalities in two provinces.176,000 ITNs will be distributed to reach universal coverage in these communities. Door-to-door registration confirms household size, the number of existing ITNs, and the number of additional nets required to ensure each household member has access to an ITN. Vouchers for free ITNs are distributed at the time of the door-to-door registration and are redeemed two weeks later at a central distribution location. Distribution is complemented by community awareness and education activities around malaria prevention and transmission. Activities include demonstrations of how to properly hang and care for ITNs. In two of the four municipalities, a hang-up campaign is being conducted in which community activists visit all households to assist hanging the nets in sleeping spaces. A post-campaign survey to assess ITN coverage and usage is planned for August 2011. Based on interim data collected, a higher use rate is expected in the two municipalities receiving the hang-up campaigns compared to those not receiving this intervention. This campaign is important as it will illuminate important barriers, challenges and opportunities that Angola's MoH can then use to design effective programming to achieve its goal of universal ITN coverage.

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LOW COST REPELLENTS FOR MALARIA PREVENTION IN RURAL AFRICA: THE JURY IS STILL OUT

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Malaria control using Long Lasting Insecticidal Nets (LLINs) is a highly effective strategy for rural Africa. However, there is growing evidence that malaria vectors are switching their feeding behavior to the early evening when people are not under their nets and are available to feed on. A cluster randomized controlled clinical trial was conducted in a village in Southern Tanzania from June 2009 to September 2010 to evaluate the additional protection provided by a 15% deet (di-ethyl toulamide) repellent lotion among LLIN users compared to LLIN users given a lotion with no deet. Consistent repellent use in the early evening may provide protection from clinical malaria episodes transmitted by early evening feeding mosquitoes. However, the power of this study was insufficiently low to draw a firm conclusion from the data. The estimate protective efficacy was 13%, lower that that expected. In order to measure this effect with sufficient power a sample size of more than 5,000 households per arm would be required. The role of repellents in malaria prevention

remains uncertain. Although there were 13% fewer clinical malaria episodes among repellent users compared to the placebo this difference did not reach statistical significance and in order to be sure that repellents are protective a much larger trial would have to be carried out. Repellents were extremely popular and the relief from nuisance biting mosquitoes was a major motivation for their use. They would need to be cheap in order to encourage uptake and strategies such as seasonal promotion prior to peak malaria season could be employed in order to maximize their potential for protection from malaria.

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IMPORTANCE OF SLEEPING ARRANGEMENT TO INCREASE BED NET USE AND REDUCE MALARIA TRANSMISSION

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A previous study found that older children tend to sleep on the living room floor without mosquito nets in villages along Lake Victoria, western Kenya. The study suggested that it is not easy for children to hang a net in a living room. We examined if this situation increases malaria transmission. A total of 849 children less than ten years old were tested for malaria infection using rapid diagnostic tests (RDT). Their caretakers were asked about bed nets use and sleeping arrangement. Of them, 530 children (62.4%) were tested positive. Nearly 70% of them did not sleep on beds, and almost half of them did not use bed nets. Older children more likely slept on the living room floor. Bed net use was lower among older children, and among children who slept on the floor. Children who slept without nets had a higher positive rate for malaria infection. Older children had a higher positive rate. When the analysis was limited to children above five years old, the result of RDT was not significantly correlated with bed net use and sleeping arrangement. The positive rate of older children was 68.7%, while that of younger children was 57.1%. These results suggest that sleeping arrangement is particularly important for younger children to prevent malaria infection.

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SOLAR-POWERED FAN PROVIDES VENTILATION WHILE SLEEPING UNDER INSECTICIDE TREATED BED NETS

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Insecticide-treated bed nets have been shown to reduce transmission of malaria by 50% in numerous endemic regions. However, many recipients are not using their bed nets due to uncomfortably hot conditions while sleeping inside of them. Here we have developed a prototype solar rechargeable fan that can be easily positioned inside the enclosed bed net space to provide ventilation and cool off the occupants. The fan features a self-contained battery pack, motor, switch, and charging circuit that allows the 9 in. long fan assembly to be plugged into the separate solar panel power source. The objective is six hours of exposure to sunlight charges to the battery pack to enable 8 hours of constant operation. The constructed prototype is a proof of concept to show that it is feasible to create a small, efficient solar powered fan. Refinements to the existing prototype will include an alternative battery pack to reduce costs, and design modifications to decrease charging time and to increase air circulation and handling.

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ENTOMOLOGICAL MONITORING OF AN INDOOR RESIDUAL SPRAYING (IRS) PROGRAM IN MALAWI

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A pilot indoor residual spray (IRS) program was initiated in Malawi in 2007 in one district with funding from the President's Malaria Initiative (PMI) and scaled up to six additional districts in 2010. Two insecticides were used, alphacypermethrin (Mokrid), and pirimiphos-methyl (Actellic). Vector abundance, insecticide decay rates and insecticide resistance were monitored to assess impact of the program. Monitoring was carried out in twenty-one villages in all the seven IRS districts. Vector abundance was monitored quarterly in three districts using pyrethrum space spray catches (PSCs) and monthly in four districts sprayed with alphacypermethrin and pirimiphos-methyl. Susceptibility tests were also carried out before spraying in selected villages in all the districts and insecticide decay rates were monitored monthly in two districts following WHO standard techniques. The main malaria vectors prevalent in the IRS districts were *Anopheles gambiae s.l* and *An. funestus s.l.* Spraying with alphacypermethrin reduced the density of *An. gambiae* to almost zero in villages where this species was predominant. There was marked reduction (>50%) in the abundance of *An. funestus* in areas where it exclusively occurred. Use of pirimiphos-methyl reduced the abundance of *An. funestus* by >90% in the areas where this species previously exhibited pyrethroid resistance. Mortality of *An. gambiae* Kisumu strain exposed onto walls sprayed with alphacypermethrin was <80% one month after spraying. On the hand, pirimiphos-methyl residues remained active for two months. Baseline susceptibility tests showed that *An. funestus* was resistant to pyrethroids (approx. 30% resistant) but susceptible to pirimiphos-methyl (100%). *An. gambiae* from Karonga District was susceptible to pyrethroids (100%). As expected, spraying with alphacypermethrin reduced populations of *An. gambiae*. The reduction observed in the population of *An. funestus* was, however, unexpected considering that the species showed resistance to pyrethroids. Use of pirimiphos-methyl resulted in marked reduction in the abundance of a previously known resistant populations of *An. funestus*. Despite these gains, the IRS program in Malawi faced a number of challenges both logistical and biological.

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FIGHTING MALARIA WITH ENGINEERED BACTERIA

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The mosquito midgut plays a key role in the malaria parasites development and subsequent transmission and also provides the habitat for diverse symbiotc bacteria. We are exploring the use of such naturally occurring bacteria as a vehicle to deliver anti-malaria effectors molecules in mosquito midgut. Specifically, we engineered *Pantoea agglomerans*, a bacterium commonly found in the midgut of *Anopheline* mosquitoes, to express a variety of effector molecules. These include 1) Salivary gland and Midgut peptide 1 (SM1), 2) phospholipase A2 (PLA2), 3) *a* single-chain immunotoxin (pbs21:shiva) composed of a single-chain antibody targeting the ookinete surface protein pbs21 and a lytic peptide Shiva-1, 4) a chitinase propeptide (Prochit) that inhibits chitinase and blocks ookinete traversal of the mosquito peritrophic matrix, 5) scorpine, a multifunctional antimicrobial peptide and 6) a *Plasmodium* enolase lysine-

rich inhibitory hexapeptide (Lrmp) that prevents plasminogen binding to the ookinete surface. By using the *E. coli* haemolysin A transport system (HlyA), the corresponding proteins were effectively secreted by transgenic *P. agglomerans* cells and accumulated in the culture media as determined by SDS-PAGE and Western-blot analysis. *In vivo* secretion of SM1 and PLA2 was confirmed by use of immunofluorescent assays that detected the binding of these proteins to mosquito midguts. Importantly, the engineered bacteria efficiently inhibited development of the human malaria *P. falciparum* in mosquitoes. *P. falciparum* oocyst counts were inhibited by 85-98%, depending on the effector gene. Significantly, the ability of mosquitoes to transmit the parasite (prevalence) was decreased by 97% for two of the effector genes (scorpine and $(Lrmp)_4$). Our findings suggest that engineered bacteria may be used to significantly strengthen existing malaria-control strategies.

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SUSTAINABLE SUPPLY CHAINS: LESSONS LEARNED FROM A LONG LASTING INSECTICIDAL NET RECYCLING PILOT PROJECT IN MADAGASCAR

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Distribution, use and timely replacement of long-lasting insecticide treated nets (LLINs) are part of a key malaria prevention strategy in Madagascar, where 5.2 million LNs were distributed from 2005-2007. There is a growing awareness of the potential environmental impact of insecticide-embedded plastic waste from the increased number of LNs, if not disposed of or recycled in an environmentally sound manner. We conducted a pilot project to collect and recycle existing old, expired LNs (oLNs) in conjunction with a mass free LN distribution campaign in November, 2010. Six health districts with an estimated population of 1.6 million were targeted for the pilot where 279,000 bed nets had been distributed in 2007. Health volunteers were trained to educate their communities, using a pre-tested job-aid, to voluntarily bring unwanted oLNs for disposal to the closest campaign community distribution point at the time of collecting their new free LNs. oLNs were collected, transported, sorted, compacted, baled and shipped to a plastics recycling company for processing. Over 22,500 oLNs were collected from 394 out of 489 (81%) community collection points. Of these, 90% were collected post-campaign. Community members were more willing to give up oLNs once the new LNs were installed in homes after the campaign distribution. Families with an insufficient number of new nets, and those using oLNs for other purposes, were reluctant to give up their oLNs. Sites with the most complex transport logistics were less likely to successfully collect oLNs. Post hoc radio messaging was found to be a useful tool to reinforce messages. The cost was $2.72/oLN collected. Costs could be substantially reduced by combining training with other LN distribution campaign preparation activities. LLINs have been successfully recycled and the material is being analyzed and tested for the most appropriate recycling use. In conclusion, collection and recycling of oLNs was found to be acceptable and feasible. Malaria programs and international donors should further explore and implement cost-effective recycling and re-use options.

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BEHAVIOR CHANGE COMMUNICATIONS (BCC) FOR MALARIA CONTROL IN SOUTHEAST NIGERIA

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Nigeria is engaged in a massive nation-wide distribution of long-lasting insecticidal nets (LLIN). LLIN ownership may be the strongest determinant of use, but there is a need for effective, evidence-based interventions to address other key determinants of net use. To inform the development of BCC strategies, The Carter Center added questions about social and behavioral determinants of LLIN use to a survey conducted in Southeast Nigeria in December 2010, prior to mass LLIN distribution campaigns. Preliminary data are presented here from 1290 individuals (43% male, 57% female) in 1192 households of Imo and Ebonyi States (2 LGAs/ state, 17 clusters/ LGA). While 83% of respondents know that malaria is transmitted by mosquitoes, 66% believe that people are only at risk for malaria during the rainy season, and 65% believe that you get malaria from eating certain foods. 72% of respondents listed protection from mosquito bites as the purpose of LLINs, but only 15% mentioned malaria prevention. Heat (15%) and allergies (5%) were rarely mentioned as disadvantages, and 42% said that LLINs have no disadvantages. 81% agreed that LLINs can be hung over any sleeping space, and 90% said LLINs are safe to sleep under. However, only 54% believe that it is safe to hang a net where you store food, and only 2.4% are aware that LLINs do not have to be re-treated. Bed nets have some negative associations: 33% think bed nets are for poor farmers, 39% think nets are "old fashioned," and 27% believe nets are part of a Western plot to reduce African populations. The data suggest that factors other than knowledge or intrinsic characteristics of LLINs may be important determinants of use (such as situational factors, norms and social support). Low literacy 46%) and limited comprehension of languages generally used for malaria communications (English 23%, Pidgin 16%), as well as limited exposure to and widespread distrust of many sources of health information, suggest that home visits conducted by trusted community members may be the most appropriate channel for malaria BCC in these areas.

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USEFULNESS OF FORMATIVE RESEARCH AS RAPID ASSESSMENT TOOL TO GUIDE IMPLEMENTATION OF INSECTICIDE-TREATED CURTAIN INTERVENTION IN IQUITOS, PERU

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As part of a community-randomized trial to evaluate the effectiveness of insecticide-treated curtains (ITC) for dengue prevention in Iquitos, Peru, formative research was conducted to assess rapidly the acceptability of ITCs in the population, and guide the research team on the approach for ITC distribution. Forty-five individuals, aged between 25 and 60 years, participated in five focus groups discussions (FGD) on the topic. After describing the curtains and passing around small ITC samples, in all five groups, all participants felt the curtains would be well accepted by themselves and people in their community. Their comments focused on how the ITC "would be favorable to families" and that it is needed

because "there are many mosquitoes in our community" or because "there is much dengue and hemorrhagic dengue around". Though overall levels of concern were low, the main one expressed related to potential allergic reactions to the ITC, particularly among children. Through the FGD we also assessed the style of curtain that people might prefer (lacey ITC was favored by all over simple bednet style because it was "more elegant"), the colors favored (light colors were preferred for most spaces, except where people would use the curtain for additional privacy), and the number of curtains people might request (median number requested was 5). The information obtained allowed us to obtain an appropriate amount and color combination of ITCs for the initiation of the trial. Also, we developed a tri-fold describing the purpose of the study, the ITCs, and providing information on how to care for the curtains, making sure to incorporate the types of concerns expressed during the focus groups. Formative research allowed us to obtain information in a rapid and cost-effective manner that was useful for the start up of our trial.

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PROMOTION OF UTILIZATION OF INSECTICIDE-TREATED NETS IN A MULTICULTURAL COMMUNITY ALONG THE THAI-MYANMAR BORDER

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This quasi-experimental study was conducted between June 2007 and April 2008, in two villages of Sangkhlaburi District, Kanchanaburi Province, Thailand. It aimed to assess the effectiveness of a health-promotion program to prevent malaria, emphasizing the utilization of insecticide-treated nets (ITN) in a multicultural community. This study applied the PRECEDE-PROCEED model for planning, implementing, and evaluating the program. It adopted four health-promotion strategies--building capacity, establishing partners and building alliances, health communication, and health education. The study was conducted in a community composed of highly diverse ethnic groups living in malaria-transmission areas along national borders. Health-promotion program activities were planned and implemented taking into account the diversity of the target population. Villagers from various ethnic groups were motivated and invited to be health-promotion volunteers. Training workshops were organized for health officers and health-promotion volunteers, to increase their capacity related to the treatment of nets and delivery of health education and health communication. The bilingual materials used for health communication and health education were co-produced by volunteers and the research team. Net re-treatment was organized twice. The effectiveness of the health-promotion program was assessed by comparing program pre- and post-test results. The results showed that the health-promotion program for malaria prevention, emphasizing the utilization of insecticide-treated nets in a multicultural community, did increase appropriate ITN use. The proportion of nets being treated and net users in the intervention group increased significantly (p value=0.00).

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BIODIVERSITY OF MOSQUITOES (*DIPTERA: CULICIDAE*) AND SAND FLIES (*DIPTERA:PHLEBOTOMINAE*) FROM THE NORTHWEST REGION OF LORETO DEPARMENT IN PERU

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From January to March 2009, mosquitoes and sand flies were collected in four villages located on the margens of the Huallaga and Maranon Rivers,in the provinces of Alto Amazonas and Datem del Maraii6n,located in Loreto Department,Peru. Collections were made using CDC light traps,human bait, and back-pack aspira tors in peridomiciliary areas. The entomologic material was kept in liquid nitrogen and transported to the Entomology Lab of NMRCD in Lima, where taxonomic identification was carried by mosquitoes and sandflies. A total of 22,513 mosquitoes were identified: 21,899 (97.27%} Culicinae and 614 (2.71%) Anophelinae. Mosquito capture ra tes were 75.28% using CDC light traps, and 17.27% using human bait, and 7.45% using back-pack aspirators. Throughout the process after collection (transport,storage,taxonomic identification), mosquitoes were preserved in cryovials at a temperature of -80°C. Biodiversity rates of *Anopheles* spp. subgenera *Anopheles, Nyssorhynchus* and *S tethomyia* were determined. *Anopheles (Nyssorhynchus)* spp. had the highest density in all collections. Eleven genera of Culicinae were identified, the *Culex* genus (with two subgenera and about 10 species identified) had the highest number of collected mosquitoes, followed by the genera *Mansonia, Ochlerotatus, Psorophoro* and *Coquillettidia.* The Shannon-Weaver diversity index was high with CDC light traps (H = 1.04), in relation to the other collection methods. In relation to sand flies,113 specimens of the genus *Lutzomyia* (77 females and 36 males) were identified, with 11species and three *Lutzamyia* spp., from which *Lutzomyia (Nyssomyia) antunesi* had the largest number of collections (64 sand flies), followed by *Lutzomyia (Nys.) yuilli yuilli* (14).

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CO-OCCURRENCE PATTERNS OF THE DENGUE VECTOR *AEDES AEGYPTI* AND *AE. MEDIOVITATTUS*, A POTENTIAL NATIVE DENGUE VECTOR IN PUERTO RICO

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Aedes aegypti is implicated in dengue transmission in tropical and subtropical urban areas around the world. *Ae. aegypti* populations are controlled through integrative vector management. However the efficacy of vector control may be undermined by the presence of alternative, competent mosquito species. In Puerto Rico, a native mosquito, *Ae. mediovittatus*, is a competent dengue vector in laboratory settings and it spatially overlaps with *Ae. aegypti*. It has been proposed that *Ae. mediovittatus* may act as a dengue reservoir during interepidemic periods, perpetuating endemic dengue transmission in rural Puerto Rico. Dengue transmission dynamics may therefore be influenced by the spatial overlap of Ae mediovittatus, *Ae. aegypti*, dengue viruses, and humans. We take a landscape epidemiology approach to examine the association between landscape composition and configuration and the distribution of each these Aedes species and their co-occurrence. We used remotely-sensed data from a newly launched satellite to map landscape features at very high spatial resolution. We found that the distribution of *Ae. aegypti* is positively predicted by urban/built-up density and by the number of tree patches, *Ae. mediovittatus* is positively predicted by the number of tree patches, but negatively predicted by large contiguous urban/built-up areas, and both species are predicted by urban/built-up density and the number of tree patches. This analysis provides evidence that landscape composition and configuration is a surrogate for mosquito community composition, and suggests that mapping landscape structure can be used to inform vector control efforts as well as to inform urban planning.

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DIFFERENTIAL EXPRESSION OF *AEDES AEGYPTI* SALIVARY PROTEOME UPON CHIKUNGUNYA VIRUS INFECTION

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Mosquito-borne diseases are excellent examples of emerging and resurging diseases that are significant global public health threats. Chikungunya virus (CHIKV) infection caused an explosive outbreak that infected as many as two million people during 2006 in India and the islands of the Indian Ocean with subsequent spread to other parts of the world. This resurging infection is transmitted primarily by *Aedes aegypti* and *Ae. albopictus*. Saliva of *Ae. aegypti* contains a complex array

of proteins essential for both successful blood feeding and pathogen transmission. Understanding salivary gland protein expression during the extrinsic incubation period of CHIKV infection is important since changes in salivary gland physiology and saliva composition could influence mosquito blood feeding success and virus transmission. CHIKV regulated mosquito salivary proteins could modulate host innate and acquired immune responses at the bite site and systemically, resulting in impaired antiviral effector functions. Using a differential proteomic approach we investigated the differential mosquito salivary protein expression during CHIKV infection. Adult female mosquitoes were fed with either CHIKV infected or uninfected bovine blood using a Hemotek membrane feeding system. Salivary glands were dissected eight days postfeeding, and proteins were extracted in 2D gel buffer. One hundred micrograms of proteins were resolved on a 2D-gel and stained with SYPRO-Ruby stain. Protein spots with a relative difference of greater than two fold, and a p-value less than 0.05 were considered a significant variation. These protein spots were excised, tryptic digested and prepared for MALDI-TOF-TOF and LC-MS-MS analysis. The expression of 22 proteins was found to be up-regulated, while 33 proteins were down-regulated. Among the up-regulated proteins, adenosine deaminase and D7 proteins have been implicated to play a major role in mosquito blood feeding. The D7 proteins belong to the family of arthropod odorant binding proteins, that facilitate blood feeding by binding to biogenic amines. These proteins are believed have anti-hemostatic and anti-inflammatory functions. Interestingly, several of the differentially expressed proteins in the salivary gland induced by CHIKV infection are proteins with unknown functions. This preliminary data establishes that CHIKV modulates mosquito salivary gland protein expression.

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BLOODFEEDING PATTERNS OF *CULEX TARSALIS* AND THE *CX. PIPIENS* COMPLEX IN CALIFORNIA

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West Nile virus (WNV) is a mosquito-borne flavivirus now endemic across several ecological regions in California. These regions are home to a wide diversity of potential avian and mammalian hosts as well *Culex* vector species. Because avian hosts have varying WNV competence, determining the bloodfeeding patterns of the *Culex* vectors is important in understanding the dynamics of virus maintenance as well as incidental transmission to disease-susceptible humans and horses. The bloodfeeding patterns of *Cx. tarsalis* and members of the *Cx. pipiens* complex were investigated from 5 locations spanning over 850km from Northern to Southern California. Nearly 100 different avian, mammalian and reptilian host species were identified from 1,487 bloodmeals using DNA sequence from a portion of the mitochondrial gene, cytochrome c oxidase I (*COI*). *Cx. tarsalis* fed on a higher diversity of hosts and more frequently on non-human mammals than did members of the *Cx. pipiens* complex when collected in the same area. Several WNV competent avian species, including House Finch and House Sparrow, were common bloodmeal sources for both vector species across several ecological regions and could account for WNV maintenance, particularly in urban settings. Highly competent Western Scrub-Jay, Yellow-billed Magpie, and American Crow also were fed upon frequently when available and are likely important amplifying hosts in some areas. The *Cx. pipiens* complex (0.4%) fed more frequently on humans than did *Cx. tarsalis* (0.2%), and horse bloodmeals were only identified from *Cx. tarsalis* (2.3%). Although neither vector species fed frequently on humans or horses in this study, with high vector abundance both species could serve as bridge vectors of WNV in several California regions.

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BARRIERS TO MALARIA ELIMINATION ON THE ISLANDS OF ZANZIBAR

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The islands of Zanzibar are the major focus of a malaria elimination campaign (defined as the reduction to zero of the incidence of locally acquired malaria). It is generally supposed that *P. falciparum* in Zanzibar is vectored by *Anopheles* species that are endophillic, anthropophagic and pyrethroid-susceptible. As a result, the islands have been saturated with permethrin or alphacypermethrin treated LLINs (long lasting insecticide treated nets) and IRS (indoor residual spraying) with lambdacyhalothrin. These campaigns have been extremely effective at reducing the prevalence of malaria to less than 1 percent. It now seems however, that the move to an elimination stage will be complicated by some recent discoveries on the ecology and behaviour of local mosquito populations. Studies conducted on the island of Pemba by the Zanzibar Malaria Control Program during 2010 and 2011, now show that most of the remaining transmission in Pemba is probably being mediated by *An. arabiensis*, and that (as a consequence of the behavioural plasticity of that species, and the high coverage of pyrethroids indoors) the majority of bites are now received out-of-door. This suggests that LLINs and IRS may need to be augmented by other control methods in order to reduce mosquito-human contact further. Moreover, a phenological characterisation of *An. arabiensis* from Pemba have shown these populations to be resistant to all pyrethroids (but susceptible to DDT, malathion and bendiocarb). The magnitude of the resistance is sufficient to markedly reduce mortality in simple bioassays against IRS residues, and used LLINs. This has prompted ZMCP and its partners to implement a change in IRS practice but with so few new vector control interventions available, or even in the pipeline, opportunities to improve upon existing control practices are very limited.

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NOVEL SOLUTIONS FOR THE DETECTION, PREVENTION AND TREATMENT OF VECTOR-BORNE DISEASES

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Florida's Schools of Pharmacy at UF and USF in concert with the UF-Emerging Pathogens Institute (EPI) and USF Center for Drug Discovery and Innovation (CDDI) and Global Health Infectious Disease Research Program (GHIDR) are developing a consortium for novel solutions for the Detection, Prevention and Treatment of Vector Borne Diseases. Vector borne diseases represent a significant health care challenge for Florida (and the tropical world), but there has been little economic incentive for the pharmaceutical industry to develop interventions. Our proposed consortium is critical to catalyze the development of efficient strategies able to solve this regional/global health-care challenge. The proposed consortium will provide a "case study" to introduce the FDA's Critical Path Initiative Development Toolkit to Florida institutions, with a focus on developing powerful scientific and technical methods such as *in vitro*, animal or computer-based predictive models, biomarkers for safety and effectiveness and new clinical evaluation techniques for a streamlined and efficient drug development as well as for establishing new validated

methods of detection and preventions. This new USF-UF consortium will place emphasis on product innovation and translational medicine and allow students and faculty to participate as team members in high profile epidemiological, drug discovery and development projects. Our consortium will "pull" and our Centers and Institutes will "push" the best emerging biomedical and biopharmaceutical technologies in Florida. Resulting infrastructure will facilitate faculty scholarship and intellectual engagement between our Universities and business and economic constituencies throughout the state and nation.

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COST-EFFECTIVE COLLABORATION BETWEEN THE UNITED STATES AND PERUVIAN NAVIES AND A PERUVIAN UNIVERSITY TO PROVIDE IMPROVED PUBLIC HEALTH MEASURES AGAINST DENGUE AND YELLOW FEVER IN PERU

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In Peru, there are no formal medical entomology programs available at the graduate or post-graduate, and very limited training at technical levels. However, Peru is endemic to many medically-important insects, including *Aedes aegypti*, which vectors dengue and yellow fever virus pathogens into human populations. An international collaboration was formed between the Peruvian Instituto de Medicina Tropical "Daniel A. Carrion" of San Marcos University (IMT DAC UNMSM), the Entomology Department of the United States Naval Medical Reserach Unit No. 6 (NAMRU-6), and the Sistema de Alerta DISAMAR of the Peruvian Navy Clinic. This collaboration resulted in the provision of formal medical entomology training specifically focused upon surveillance and control of *Aedes aegypti*, the mosquito vector of dengue and yellow fever viruses, to Peruvian naval Nurses, who will be stationed in remote locations throughout Peru during their Naval careers. This collaboration has been organized as a long-term collaboration, with the goal of providing this training 2-3 times each year to new active-duty Peruvian nurses prior to their deployment to remote areas in Peru that are endemic to these debilitating diseases.

1244

CHANGES IN RELATIVE ABUNDANCE OF *ANOPHELES GAMBIAE* S.S. AND *AN. ARABIENSIS* IN SUBA DISTRICT, WESTERN KENYA: ITS RELATION TO BED NET COVERAGE

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Coverage of insecticide treated bed net has increased considerably in Kenya for the past few years. Since insecticide treated nets kill indoor mosquitoes, the relative abundance of *Anopheles gambiae* s.s. to *An. arabiensis* may decrease, because *An. gambiae* is more endophilic and anthropophilic. We compared the current relative abundance of both species with that in the past in Suba District. Then, we examined the relationships between relative abundance and bed net coverage. Anopheline larvae were collected from the same areas in 2009 and 2010 that were surveyed by a study in 1998. Indoor resting anophelines were also collected in the same villages in 1999 and 2008. Moreover, we monitored the relative abundance and bed net coverage periodically from 2007 for three years. In the larval survey, over 90% of collected larvae were *An. arabiensis* in 2009 and 2010 while approximately 70% were this species in 1998. The density of indoor resting anophelines in 2008 was one seventh of that in 1998. The decrease was mainly due to the decrease of *An. gambiae* s.s., which increased the relative abundance of *An. arabiensis* from 9.3% to 39.2%. The three-year survey revealed non-linear relationships between bed net coverage and relative abundance of *An. arabiensis*. When coverage exceeded 0.7 nets per person, the density of *An. gambiae* s.s. decreased, and the relative abundance of *An. arabiensis* increased. However, the trend was unclear below 0.7 nets per person. The results support the notion that bed net coverage alters the relative abundance of malaria vector species. In an area where *An. arabiensis* is dominant, the effectiveness of bed nets may be hampered.

1245

LA CROSSE ENCEPHALITIS IN EASTERN TENNESSEE: EVIDENCE OF INVASIVE MOSQUITO (*AEDES ALBOPICTUS* AND *OCHLEROTATUS JAPONICUS*) INVOLVEMENT IN THE TRANSMISSION OF AN INDIGENOUS DISEASE

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La Crosse encephalitis virus (LACV), family Bunyaviridae, is an important cause of pediatric encephalitis in the United States. The virus is transmitted by the bite of infectious mosquitoes, primarily the native tree- hole mosquito *Ochlerotatus triseriatus*. Since being characterized in the 1960s, human cases have been concentrated in the upper- Midwestern states where the virus is considered endemic. Approximately 80- 100 cases are reported annually. While death is rare, symptoms can be severe and often require hospitalization. In the mid 1990s, a new focus of the disease was recognized in West Virginia, North Carolina and eastern Tennessee. One hypothesis for the establishment of this new focus is that the invasive mosquito, *Aedes albopictus*, may be acting as a novel vector in this area. A third mosquito species, *Oc. japonicus*, has recently become established in the region and is also a competent vector of LAC in the laboratory. The potential for invasive mosquitoes to modify disease epidemiology is large. These three species occupy many of the same larval habitats and the invasive species may have an effect on the local mosquito community due to resource competition. To test the invasive vector hypothesis, mosquito eggs, larvae, and adults were collected weekly from six recent human case sites in eastern Tennessee from May - August 2010. Three pools of *Ae. albopictus*, one pool of *Oc. japonicus* and eight pools of *Oc. triseriatus* were LACV positive by PCR. Additionally, eleven of the twelve positive pools came from mosquitoes collected as eggs, indicating active transovarial transmission. This is the second study to find field caught mosquitoes positive for LACV in Tennessee with the first sample being *Ae. albopictus* from 1999. To our knowledge, this is the first recorded report of *Oc. japonicus* being naturally infected with LACV and in close association with human habitation. This study provides further evidence that invasive species may have changed the epidemiology of a vector-borne disease in the United States. Viral assays are ongoing.

1246

INVESTIGATIONS INTO MOSQUITO BLOOD FEEDING PATTERNS ON WILDLIFE AND A POTENTIAL ROLE FOR BATS IN ARBOVIRUS TRANSMISSION CYCLES IN UGANDA

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Zoonotic and vector-borne pathogens have comprised a significant component of emerging human diseases in the last decade. Uganda has a history of enzootic and epizootic arbovirus activity and has been predicted as a hot spot for disease emergence. Serological evidence

exists documenting exposure of various East African bat species to many arboviruses including Rift Valley fever, Yellow fever, West Nile, Usutu, Sindbis, Bunyamwera, and Zika viruses, however the role of bats in arbovirus transmission cycles is poorly understood. While collecting mosquitoes as part of an emerging arbovirus surveillance project in Uganda, we obtained blood-engorged *Culex* mosquitoes which had fed on fruit bats in both Semliki and Maramagambo Forests. To follow up on these observations and investigate the role of bats in arbovirus transmission cycles, blood samples from *Rousettus aegypticus* bats collected from the python cave in Maramagambo Forest were screened for West Nile, Yellow Fever, Dengue, Chikungunya, and O'nyong'nyong viruses by plaque reduction neutralization test (PRNT), and mosquitoes were trapped from around the vicinity of the cave. Blood and tissue samples were also collected from various fruit and insectivorous bat species in Kampala, Uganda and tested for evidence of arbovirus infection by PRNT and virus isolation. Serological and virological evidence will be presented on the arbovirus exposure history of several species of bats in Uganda. The blood feeding patterns of mosquitoes on a diversity of wildlife species in Uganda and potential enzootic arbovirus transmission cycles between mosquitoes and wild vertebrates including bats will be discussed.

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LANDSCAPE ECOLOGY OF DENGUE AND CHIKUNGUNYA SYLVATIC VECTORS IN SOUTHEASTERN SENEGAL

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Dengue (DENV) and chikungunya viruses (CHIKV) circulate in a sylvatic transmission cycle between non-human primates and arboreal Aedes spp. in Kedougou, Senegal, and several studies have shown a low incidence of infection by both sylvatic viruses in humans in West Africa as well. Although humans are probably infected by sylvatic vectors, the extent and mechanisms of contact between humans and sylvatic vectors remains unknown. To gain insight into the role of different mosquito species in both enzootic transmission in primates as well as spillover into humans, between 2009 and 2010 we monitored the distribution of a broad array of mosquito species in five landscape classes (forests, savannahs, barren, agricultural, and villages) in the Kedougou area. Mosquito were collected monthly in each of the landscape classes from 18:00 to 21:00 hrs and identified to species. Among 39,799 mosquitoes collected, the most and least abundant species were *Ae. vittatus* and *Ae. aegypti*, respectively. The abundance of *Ae. vittatus, Ae. luteocephalus* and *Ae. aegypti* peaked in June, while that of other species peaked twice between July and November, 2009. The preferred habitat of *Ae. africanus, Ae. luteocephalus* and *Ae. taylori* was the forest canopy, while the others species were distributed more evenly across the five landscape classes. CHIKV was detected by real-time PCR assay and/or virus isolation in 39 pools of mosquitoes, including previously recognized (*Ae. furcifer, Ae. taylori, Ae. dalzieli, Ae. luteocephalus, Ae. africanus, Ae. aegypti, Ae. neoafricanus*, *Ae. hirsutus, An. funestus, An. coustani*, *Ma. uniformis*) and potentially new (*Ae. metallicus, Ae. centropunctatus, Ae. hirsutus, An. domicola* and *Cx. poicilipes*) CHIKV vectors. Infection rates showed temporal and spatial variation. No DENV was detected. Our findings provide insight to the ecology of sylvatic vectors of DENV and CHIKV in a changing environment affected by urbanization and deforestation associated in part with mineral exploitation.

1248

EVOLUTIONARY HISTORY OF *AEDES AEGYPTI*: A GLOBAL PERSPECTIVE

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Aedes aegypti is the principal vector of both dengue and yellow fever viruses worldwide. A human commensal, this mosquito species has successfully invaded much of the tropical and subtropical world over the past few centuries. Though *Ae. aegypti* is often treated as a homogenous species, populations of the mosquito differ markedly in their association with human habitats, as well as in their ability to transmit dengue viruses. Recent microsatellite work in our lab suggested that the African sylvan subspecies, *Ae. ae. formosus*, is ancestral to the worldwide domestic form (*Ae. ae. aegypti*), but that close human association has likely evolved multiple times independently in *Ae. aegypti*. In order to more formally test hypotheses of ancestry and trait evolution, we sequenced 4 variable nuclear loci from 167 individuals representing 17 global populations of *Ae. aegypti*. The same regions were sequenced in two closely related species to provide outgroups for rooted phylogenies. In addition, a sequenced RAD (restriction-site associated DNA) approach was undertaken to explore at a fine-scale the history and colonization of *Ae. aegypti* out of Africa and across the global tropics and subtropics. This method allows simultaneous detection and screening of thousands of SNPs across the *Ae. aegypti* genome. Bar-coded RAD libraries were successfully constructed from 136 individual mosquitoes (8 each from 17 populations) and sequenced on an Illumina platform. Both the 4 sequenced nuclear loci and the RAD markers confirm African *Ae. ae. formosus* as the ancestral form of the species, and support multiple "domestication" events. However, the RAD markers are significantly more sensitive at detecting population structure and tracing the invasion history of this important vector arthropod out of Africa and across the world. In addition, the SNPs detected in our RAD analyses will prove useful in future association mapping studies, such as those for important epidemiological traits including vector competence for dengue and human host preference.

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HOST ATTRACTION OF ANOPHELINES IN SOUTH HALMAHERA, INDONESIA

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The feeding behaviors of Indonesian malaria vectors remain largely uncharacterized. A Latin square design was used to compare anophelines attracted to human, cow, and goat-baited tents. The experiment was carried out for 12 nights in August 2010 in Saketa village in South Halmahera, Indonesia. Specimens were collected from the inside walls of baited tents every hour from 18:00 to 7:00 hours and were morphologically identified. A subset of bloodfed specimens were analyzed using a bloodmeal diagnostic PCR assay. 1,235 *Anopheles* specimens of nine different morphological species were collected over 12 catch nights. These morphological species included *An. farauti, An. hackeri, An. indefinitus, An. kochi, An. punctulatus, An. subpictus, An. tessellatus, An. vagus*, and *An. vanus*, all of which have been previously shown to be capable of transmitting *Plasmodium* parasites. 1024, 137, and 74 anophelines were collected in cow, goat, and human-baited tents, respectively. Bloodmeal analysis of specimens collected in the human-baited tent indicate a low level of multiple host blood feeding. Morphological species distribution was similar between the cow and goat-baited tents, with a majority (44% and 36%) of *An. indefinitus*,

but different for the human-baited tent, with a majority (41%) of *An. vagus*. Eight of the nine morphological species represented in this study were captured on each of the three hosts, suggesting a plasticity in host attraction behavior. Multiple host feeding and flexibility in feeding behavior could have important implications for malaria control.

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ASPECTS OF ECOLOGY OF POTENTIAL RIFT VALLEY FEVER VIRUS MOSQUITO VECTORS, KHARTOUM STATE, SUDAN

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Rift valley fever epidemics are disruptive and expensive to local and regional economies. After a devastating outbreak of Rift Valley Fever in khartoum state, Sudan 2007; ecological baseline surveys were conducted in Khartoum State, Sudan, during the rainy season (end of July to the beginning of September) 2008 in order to identify mosquito species present and evaluate their emergence and survivorship. Larval identification of species of Culicine and Anopheline mosquitoes present in Khartoum State taken from five study sites represents Khartoum state indicated that *Anopheles arabiensis* is the only species of the Anopheline mosquitoes found. Three species of culicine mosquitoes were found: *Culex quinquefasciatus*, *Cx univittatus* and *Cx arbeeni*, Species of *Aedes* were found in irrigated schemes at one study site and was absent from the other four study sites, these species were *Ae. vittatus* and *Ae. vexans*, whose presence was recorded after the onset of the rainy season.the same breeding site was first occupied by *Ae.vittatus* then *Ae. vexans,* with an interval of habitat drying. Daily emergent adults Culicine and Anopheline mosquitoes present were taken from randomly selected breeding sites in the five study sites, population measurements were performed. The absolute number of emergent adults was obtained by collecting mosquitoes under net-traps covering the breeding sites. Records were taken each day for seven constitutive days, synchronized emergence of males and females was observed at all the study sites, showing an overall marked predominance of females in emergence trap catches. Adult survival rate was the most important factor determining the stability of the population and total egg production. Females that become infected when taking a blood meal must survive throughout the incubation period of the pathogen. Under controlled laboratory environment, effect of food types (sucrose 10%, sucrose 10% and blood diet) on longevity of adult female mosquitoes was conducted, sugar-fed and blood-fed mosquitoes exhibited very high percentage of surviving rates beyond the 15 days (incubation period for RVFV),However these have varied among the five study areas. Also results indicated prolonged survival of sugar-fed female mosquitoes more than blood and sugar fed females, this served to increase survivorship of females until they find the appropriate host.

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MAIN MOSQUITO BREEDING SITES FOR *AEDES AEGYPTI* IN THE PAN-AMERICAN HIGHWAY: CUCUTA-PAMPLONA AREA (NORTE DE SANTANDER - COLOMBIA) IN 2010

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Aedes aegypti is the principal dengue vector in Colombia where dengue transmission is limited by the presence of the vector; unfortunately in this country, the presence of *Ae. aegypti* has been documented up to 2200 m.a.s.l. Norte de Santander is the second most endemic area for dengue in the country. Previous studies have associated travel and transport as key factors in the spread of diseases and vectors. With this pilot study, we investigated the main breeding sites and mosquito larva species on the highway from Cucuta (325 m.a.s.l) to Pamplona (2342 m.a.s.l) in 75km distance. We found that tires where the main breeding site followed by plastic containers and small pools along the way. The main specie collected was *Ae. aegypti* followed by *Culex quinquefasciatus*. *Anopheles* mosquitoes were not found in the highway area. Tire repair shops were the places with the highest number of infected tires; we also found abandoned tires infected with mosquito larva.

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A COMPARATIVE EVALUATION OF SIX DIFFERENT MALARIA VECTOR COLLECTION METHODS IN LOW-LYING MALARIA ENDEMIC REGIONS OF WESTERN KENYA

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Outdoor biting and other forms of behavioral adaptation by malaria vectors to domestic insecticide-based control measures may compromise the sensitivity of conventional sampling tools operating indoors such as light traps or indoor resting catches, thus preventing effective surveillance and management of vector populations. We evaluated six different vector collection methods to optimize a protocol for operational sampling of malaria vectors robust to variations in vector behavior, notably variations associated with the presence of important malaria control methods. Over 30 days, we replicated a Latin square design 10 times at sites in 4 districts in western Kenya: Kisumu, Bondo, Nyando and Rachuonyo. Each site consisted of 3 locally representative houses through which the six different sets of trapping methods were rotated every 3 nights in a random order of three possible arrangements: 1) Indoor human landing catch (HLC) and outdoor HLC, 2) CDC Light trap placed beside an occupied insecticide-treated net indoors combined with Ifakara tent traps outdoors, and 3) Window traps to catch exiting mosquitoes combined with both pot and box formats of resting traps placed both indoors and outdoors. At each site, a fourth house was selected for pyrethrum spray catch (PSC). The top collection methods with their corresponding number of *Anopheles* per collection effort were PSC (10.5), HLC indoor (3.0), Light trap (3.0) HLC Outdoor (2.8) and Ifakara tent traps (2.7). Resting Boxes and Pots positioned both indoors and outdoors caught less than 1 *Anopheles* per collection effort. HLC outdoor collected the highest amount of *Culex* at 77.4 per collection effort. Irrespective of the intensity or type of insecticide based vector control method in place and of biting behavior of the local malaria vectors, we conclude that pyrethrum spray catch is the most sensitive method for vector collection in low lying malaria endemic regions of western Kenya.

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HETEROGENEOUS FEEDING PATTERNS OF *AEDES AEGYPTI* IN HOUSEHOLDS IN IQUITOS, PERU

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Heterogeneous biting by female mosquitoes can significantly alter transmission of mosquito-borne pathogens. Previous studies show *Aedes aegypti*, the primary vector of dengue viruses (DENVs), more frequently bite individuals with higher body mass index (BMI). Because BMI increases with age, we expect positive linear relationship with age and biting. Studies show, however, that young adults receive more bites than older adults. Factors such as sex, mosquito exposure time and previous DENV infection should, therefore, be used to analyze heterogeneous feeding patterns. Between October 2009 and November 2010, 2,035 interviews with 280 participants were conducted in 19 households in Iquitos, Peru.

Interviews focused on anthropomorphic characteristics and time spent in houses. In the week following interviews adult mosquitoes were collected twice daily, yielding 1,878 engorged and partially engorged mosquitoes. Engorged abdomens were excised and participant DNA was obtained by cheek swab. All DNA was extracted using Qiagen extraction columns. Human DNA was amplified at 10 microsatellite loci, and allelic profiles identified using capillary electrophoresis. A computer program matched participant profiles to mosquito blood meals. To date, 99 of 115 identified blood meal profiles have been matched to participants. 29 young adults (ages 15 - 35) received 50 bites (1.72 bites/person). 14 children (<15) and 23 older adults (>35) received 154 and 34 bites, respectively (1.07 and 1.48 bites/person). In one household of 12 residents ranging in age from 5 to 70 years with BMI of 13 to 32 kg/m^2, 2 young adults ages 27 and 31 with BMI's of 23 and 21.2 contributed to 46% of the 26 identified blood meals, consistent with the idea that young adults are bitten most often, and indicating that age better predicts biting frequency than BMI. Analysis of the remaining 1,763 mosquitoes and interview data will be completed in the next 4 months. Results will be used to model virus transmission and to compare various vaccine delivery strategies.

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WHOLE GENOME SEQUENCING OF *ANOPHELES PUNCTULATUS* SIBLING SPECIES OF PAPUA NEW GUINEA

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The *Anopheles punctulatus* (AP) group in Papua New Guinea and Southwest Pacific consists of at least 13 sibling species that include the vectors of malaria and lymphatic filariasis. Understanding the population organization of the mosquitoes as well as the molecular basis for the phenotypic variability related to vector competence or control is complicated by limited data on the genetic diversity of these mosquitoes. We present here data generated by whole genome sequencing from individual AP mosquitoes and show that this approach provides extensive catalogues of genetic polymorphisms and can significantly contribute to better understand the biology of these mosquitoes. We extracted DNA from individual mosquitoes, and after determination of the species status by species-specific PCR-based assay, sheared the DNA molecules into 250-300 bp fragments and prepared libraries for two *Anopheles punctulatus* mosquitoes, one *An. farauti* 1, one *An. farauti* 2 and one *An. koliensis.* We sequenced each library on individual lanes of an Illumina GAIIx (paired-end 51 bp) or HiSeq 2000 (paired-end 100 bp). Overall, less than 1.5% of the reads generated could be mapped to the *An. gambiae* (AG) reference genome sequence suggesting that the sequence divergence between AP and AG is too great for the latter to serve as a useful reference sequence. We therefore reconstructed large chromosomal segments ("contigs") using solely the sequence information contained in the reads. Using this procedure we successfully assembled the entire mitochondrial genome sequence for each of the five mosquitoes which confirmed the deep divergence between AP and AG but also revealed deep divergences among the AP sibling species. In addition, we assembled 50-60% of each genome into fragments larger than 1,000 bp and identified more than 40,000 DNA polymorphisms that can now be used in association studies for traits related to insecticide resistance, preference to human blood meal or capacity to transmit malaria and filariasis.

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A COMPUTER SYSTEM FOR FORECASTING WEST NILE VIRUS RISK USING EARTH OBSERVATION DATA

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Although there have been many calls to expand the use of earth observation technologies in the health sciences, there are few examples of operational systems with demonstrated impacts on public health. Our research objective was to bridge the gap between remote sensing and public health by developing decision support systems to provide health scientists and practitioners with access to environmental information for surveillance and forecasting of mosquito-borne diseases. Specific objectives were to automate the processing of remote sensing data to generate environmental metrics, analyze the predictive capabilities of these metrics using retrospective datasets of human disease cases, and develop a web-based system for visualization and analysis of the resulting products. The system was programmed using JAVA for user interface development and overall system control. Spatial analyses were carried out using Python scripts to call ArcGIS geoprocessing functions. PostgreSQL was used for the storage and manipulation of the resulting data summaries. We implemented a prototype of the system to forecast outbreaks of West Nile virus in the northern Great Plains. Environmental variables included MODIS land surface temperature (LST) and vegetation indices (e.g., NDVI, EVI) derived from the MODIS nadir BRDF-adjusted reflectance product. We also used these data to compute actual evapotranspiration (ETa) using the simplified surface energy balance method. Statistical analysis using generalized additive models (GAMs) revealed non-linear associations between interannual variability in WNV incidence and interannual deviations of cumulative LST, NDVI, and ETa throughout the spring and early summer. There was an early-season influence of the timing of spring onset (captured by NDVI) as well as a late spring/summer influence of accumulated moisture and temperature (captured by LST and ETa). Forecasts are currently being disseminated via a web atlas (http://globalmonitoring.sdstate.edu/eastweb) and will be validated using surveillance data from the 2011 WNV season.

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DYNAMICS OF *ANOPHELES GAMBIAE* POPULATIONS IN THE SAHEL: NEW PATTERNS AND NEW PUZZLES AWAIT NEW UNDERSTANDING

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Malaria remains a top public health priority across Sub-Saharan Africa, where it is transmitted primarily by *Anopheles gambiae s.s.* and *An. arabiensis*. Populations of these species exploit diverse environments including dry savannahs and semi-desert areas, where surface waters required for larval development are absent for large parts of the year. How mosquitoes survive the long dry season has been debated without resolution for over 60 years. Although recent studies provide evidence for aestivation (extended survival throughout the 4-7 month-long dry season) of M form *An. gambiae*, the role of long-distance migration from areas with year-round breeding remains unclear. Here, we analyze the dynamics of the members of the *An. gambiae* complex in the Sahelian village Thierola (Mali), focusing on the dry season and its preceding and subsequent transition periods, over a period of three years (2008-2011). The dry season mosquito populations were characterized by low overall density (<0.05 mosquito/house), and were predominantly composed of

the M form (>95%), with the remainder being *An. arabiensis*. Males were found throughout the dry season, both indoors and in swarms, albeit in very low numbers. Interestingly, the dry-season dynamics were not stable: in early April, ~2 months before the first rain, density surged up to three orders of magnitude and receded to typical dry-season density within days. This surge was observed in both 2010 and 2011 and consisted only of the M form. Five to seven days after the first rains (early June), before a new generation of adults could be produced, the M form surged again over one order of magnitude, and continued to increase gradually at an average rate of 50%/week, for several weeks. Unlike the M form, the S form and *An. arabiensis* remained virtually zero for over four weeks after the first rains; thus it is unlikely that they aestivated but would emerge only ~5 weeks after all larval sites filled. These results suggest that both the S form and *An. arabiensis* persist in the Sahel primarily by migration whilst the M form aestivate. Final analysis and implications for malaria control will be presented.

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DIRECT AND INDIRECT COSTS OF *PLASMODIUM* INFECTION ON MOSQUITO REPRODUCTIVE SUCCESS

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Infection with malaria parasites reduces the immediate reproductive success of mosquitoes, but the life-long effects, as well as their interaction with stress, are not well known. Additionally, the negative effects of infection may be exacerbated by the nutritional cost of feeding on anemic blood. We evaluated the effect of *Plasmodium gallinaceum* infection on reproductive success of stressed and unstressed *Aedes albopictus*, fed on either infected or uninfected chicken blood. Each of these treatment combinations were subdivided into three subgroups that were either fed: (i) directly on an infected (or uninfected) chicken (Live); (ii) membrane-fed on fresh blood from the same chicken (Mem_{FRESH}); (iii) membrane-fed on the same blood incubated at 4°C for 12 h (rendering infectious blood non-infectious; Mem_{UNINF}). The mosquitoes were subsequently fed two more times on uninfected blood from the same chicken. The egg batch size (EBS) of individual mosquitoes was determined 7 d after each feed. Preliminary analyses revealed that EBS was lower in infected vs. uninfected and stressed vs. unstressed mosquitoes. However, the interaction between stress and infection was not significant. Likewise, there was no significant interaction between infection and feeding type (i.e. Live, Mem_{FRESH}, and Mem_{UNINF}), indicating that the fitness costs of being fed on an infected chicken were similar in both infected-infectious (Mem_{FRESH}) and infected-non-infectious (Mem_{UNINF}) blood. We also found that the negative effects of infection and stress on EBS were not restricted to the first oviposition cycle, but rather that these factors could lead to a dramatic decline in the lifelong reproductive success of individuals. Our results highlight both the life-long and indirect (i.e. due to anemic blood) fitness costs of *Plasmodium* infection to both stressed and unstressed mosquitoes. Such costs are important from an ecological and epidemiological perspective, as they could affect evolution of resistance/tolerance mechanisms, and in turn affect mosquito population dynamics and vector potential.

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MOSQUITO COMMUNITIES AND VECTOR-ASSOCIATED MICROBIOMES SAMPLED ACROSS A HABITAT GRADIENT OF THAILAND

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Changes in biodiversity have the potential to affect the risk of infectious diseases in plants and animals, including humans, since infectious disease distribution is largely dependent inter-specific interactions. In particular, mosquito-borne diseases are well-suited to study how changes in interacting species, namely mosquitoes, their hosts, and associated microorganisms in changing habitats may affect infectious disease risk. Current knowledge of mosquitoes and their associated microbial communities in natural habitats is, however, limited. Here we explored the composition and diversity of mosquitoes and mosquito-associated microbes in relation to habitats ranging from forest to urban areas in the central plain of Nakhon Nayok province, Thailand. During the rainy season in 2008, adult mosquito collections from 24 sites using CDC light traps, BG sentinel traps, Mosquito Magnet traps, and CDC backpack aspirators yielded a total of 62,511 identifiable female mosquitoes of 54 confirmed taxa. Female mosquito abundance was highest in the rice field habitat and lowest in the forest habitat with 27,041 (43.26%) and 4,840 (7.74%) mosquitoes collected, respectively. The diversity of mosquito communities was characterized using a variety of diversity measurements including statistical sampling approaches to extrapolate species richness. In general, the rural habitat was the most diverse while the least diverse habitat varied depending on the indices used. The Vishnui subgroup of Culex species was the most common taxon found overall and also the most common in the fragmented forest, rice field, rural, and suburban habitats, while Uranotaenia sp. was the most common taxon in the forest habitat and *Cx. quinquefasciatus* was the most common species in urban settings. *Aedes aegypti* and *Ae. albopictus* were most abundant in urban and rural area respectively. To explore the diversity and composition of vector-associated microbiomes, the microbiota from three vector species *Cx. quinquefasciatus*, *Ae. aegypti*, and *Ae. albopictus* from different habitat types were studied using 454 pyrosequencing of ribosomal RNA. Patterns of microbiota community assembly in mosquitoes by habitat type and vector species using both alpha- and beta-diversity analyses will be discussed. Our results are particularly relevant for understanding the dynamics of mosquito vectors and their associated microbiomes in landscapes of Thailand.

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LINKING OVIPOSITION-SITE CHOICE TO OFFSPRING FITNESS IN *AEDES AEGYPTI*: CONSEQUENCES FOR TARGETED LARVAL CONTROL OF DENGUE VECTORS

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Maternal oviposition-site choice and its repercussions for offspring fitness are known to influence population dynamics of insects. Using four experimental container treatments (size [large vs. small] x water management [manually filled vs. unmanaged]), we tested the hypothesis that wild *Aedes aegypti* in Iquitos, Peru choose egg-laying sites to maximize offspring survival and growth. Among 80 containers located

in 20 houses, females consistently laid more eggs in large vs. small containers ($\beta = 9.17$, $p < 0.001$), and in unmanaged vs. manually filled containers ($\beta = 5.33$, $p < 0.001$). There was poor correlation, however, between oviposition preference and two components of mosquito fitness, pupation probability and adult size. Probability of pupation was higher for mosquitoes developing in small, unmanaged containers than any other container type ($\beta = 3.4$, $p < 0.001$). Adult body size decreased for individuals developing in large containers (females: $\beta = -0.19$, $p < 0.001$; males: $\beta = -0.11$, $p = 0.002$) and unmanaged containers (females: $\beta = -0.17$, $p < 0.001$; males: $\beta = -0.11$, $p < 0.001$). Our data suggest that the majority of *Ae. aegypti* eggs are laid in non-optimal sites, such that selective oviposition behavior contributes to population regulation by limiting the production and size of adults. Targeted larval control strategies removing the most productive containers may have the unintended effect of encouraging females to spread their eggs more evenly among remaining containers. By tracking egg-laying patterns of individual females inside a semi-field enclosure, we found that the probability of any container receiving eggs increased when preferred container were removed (but the total number of containers remained constant) ($\beta = 1.36$, $p < 0.001$). We suspect that in Iquitos, and possibly other locations, selective oviposition behavior by *Ae. aegypti*, along with a potential switch from clustering eggs to spreading them out, will render targeted larval control less effective than anticipated.

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TOWARDS A CONSERVED *CIS*-REGULATORY MODULE WITH CROSS-STRAIN/SPECIES APPLICATION FOR DRIVING ANTI-PATHOGEN EFFECTOR TRANSGENES: COMPARATIVE TRANSCRIPTOMICS TO DISCOVER EARLY BLOODMEAL-RESPONSIVE, CIS-REGULATORY SEQUENCES FROM MOSQUITO MIDGUT RNA-SEQ

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Empirical definition of active *cis*-regulatory elements (CRE) through classical "promoter bashing" is difficult in mosquitoes due to the time and effort required to produce transgenic mosquito strains. Bioinformatic methods combined with existing biological knowledge and quality mRNA abundance data should allow the inference of active CRE combinations, *cis*-regulatory modules (CRM), without requiring construction of transgenic mosquito strains. The ecdysone (20E) response cascade is conserved throughout insects and has been shown to drive changes in mRNA abundance following the ingestion of a bloodmeal. This supports the hypothesis that it should be possible to deduce a conserved CRM by studying bloodmeal-regulated transcript abundance across multiple mosquito species. 20E has had multiple early-response factors described previously including the ecdysone receptor (EcR), its binding partner ultraspiracle (USP), and the 20E-inducible gene E74. Other laboratories have shown that levels of 20E early-response factor isoforms vary in a time- and tissue-specific fashion in response to pulses of 20E following a bloodmeal. This allows one hormone to regulate diverse cellular responses. Tissue-specific, time-course RNA-Seq data with high temporal resolution (2 hours) will be used to compare 20E early-response factor isoform mRNA expression levels across evolutionarily distant species (*Anopheles gambiae, Aedes aegypti, and Culex quinquefasciatus*) to infer transcripts that display probable time-lagged induction by 20E and harbor known 20E early-response factor motifs. This transcript set will leverage a combined comparative-genomics and expression-profile based CRE/CRM discovery strategy to reveal putative CRMs expected to provide a better understanding of 20E-regulated transcript regulation in the midgut. The discovered CRMs will serve as the basis for validation of a set of conserved CREs that may be combined to drive robust anti-dengue effector transcription in the midguts of *Ae. Aegypti* mosquitoes directly following the ingestion of each bloodmeal.

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USING MOSQUITO SURVEILLANCE DATA TO PREDICT HUMAN WEST NILE VIRUS TRANSMISSION RISK

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West Nile virus (WNV) has become endemically established across the Americas with enzootic activity and significant human illness. Despite this, funds for surveillance and control are limited and decreasing. Predicting the risk of human infection to initiate timely preventative measures is the primary goal of public health mosquito surveillance. Many arbovirus response plans outlines recommended public health and mosquito control actions based on levels of virus activity determined by statewide mosquito surveillance. However, studies linking mosquito surveillance data to the spatio-temporal risk of human WNV infection, have rarely been attempted. We quantified the links between mosquito surveillance data and the spatio-temporal patterns of 3,827 human WNV cases reported in Colorado from 2003-2007. Mosquito data were strongly predictive for spatio-temporal variation in human WNV infections several weeks in advance in a statewide analysis, and with temporal variation within a county. Correlative and predictive relationships were strengthened by using pooled estimates of prevalence from across the state in estimating of risk early and late in the season when few mosquitoes were trapped at the local scale. However, we found that when current year prevalence data was not available, as could occur with reduced or eliminated surveillance budgets, no meaningful predictions of human risk could be made to determine appropriate public health response. Overall, our results demonstrate that mosquito surveillance provides valuable predictive data about the risk of human infection which can be used to trigger emergency response actons and allocate limited public health and mosquito control resources.

1262

DOCUMENTING THE POTENTIAL INTRODUCTION OF DENGUE VIRUS INTO KEY WEST, FLORIDA THROUGH AIRLINE AND CRUISE SHIP PASSENGERS FROM DENGUE-ENDEMIC LOCATIONS

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For the first time in decades, sporadic cases of locally-acquired dengue were reported in Key West in 2009 and again 2010. Current hypotheses regarding this continuance include vertical transmission, the establishment of an endemic state with undetected transmission between years, and multiple introductions via visitors from endemic countries during both years. Regarding the third hypothesis, country- and year-specific dengue incidence data (PAHO) and the numbers of airline passengers originating in dengue-endemic countries in this hemisphere with a final destination of Key West were used to estimate the relative the magnitude of potentially viremic passenger-days experienced per year. These estimates suggest multiple introductions per year are not uncommon and that potential introductions in 2009 and 2010 were higher than in 2007 and 2008 as a result of an increase in air travel and major dengue activity in the Caribbean and Central America. Both years were El Niño years that historically are associated with elevated temperatures and higher dengue activity in the region. A similar analysis of potential introductions via the cruise ship industry will also be presented.

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CRYPTIC BREEDING: A POTENTIAL CAUSE OF LOCAL DENGUE TRANSMISSION IN KEY WEST, FLORIDA

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June 2009 marked the beginning of a 2-year outbreak of locally-acquired dengue in Key West, Florida. Despite increased control efforts by mosquito control and local residents, the number of dengue cases in 2010 nearly doubled that of 2009. Surveillance on the abundance of immatures was inconsistent with magnitude of the adult population of *Aedes aegypti*. Similar disconnects(?) between immature and adult abundance in other dengue-endemic regions have been the result of cryptic breeding which occurs when mosquitoes reproduce in locations that escape control efforts. The majority of homes in Key West were built prior to municipal utilities and stored water in cisterns and disposed of waste through septic systems. Cisterns and unused septic tanks are several cubic meter in size and most are not easily accessible. Though historical maps exist, the true number of cisterns and septic tanks is unknown thus complicating control efforts. Presented here are the combined efforts of the University of Florida and Monroe County Mosquito Control to identify and eliminate cryptic breeding sources for *Ae. aegypti* in Key West.

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THE QUALITY OF DRINKING WATER IN COMMUNITIES ALONG THE MARANON RIVER IN THE PERUVIAN AMAZON

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Water is one of the world's most critical resources, however international water quality surveillance and monitoring is often not implemented, obscuring associations and etiologies of potentially related illnesses. We conducted an evidence-based approach to understand the sources and types of water contaminants as well as the overall safety of available drinking water in Peru. A comprehensive, portable, water quality assessment toolbox was used to quantify key microbial (total coliforms, *E.coli* and enterococci) and chemical (metals, anions and pesticides) contaminants. This assessment system was applied in the field to evaluate the drinking water of 20 rural villages bordering the Maranon River in the Peruvian Amazon. In total, 32 households, 32 drinking water sources, and 2 water treatment systems were assessed. All household drinking water samples and 93% of source water samples contained moderate to high levels of *E.coli* contamination. Water treatment systems varied in contaminant removal, ranging from 2.03 logs to 4.15 logs of measured bacterial removal. Multiple water samples contained chemical contaminants in excess of WHO guideline levels including phosphate (anion); aluminum, iron, and manganese (metals); and lindane (pesticide). Current international water quality screening and evaluation efforts are not adequate to address the burdens caused by the adverse health effects of waterborne contaminants, thereby demonstrating the need for portable water quality screening. In the Peruvian Amazon, results comparing source water and household contamination suggest recontamination during transport. Analysis of chemical pollutants revealed a need for water treatment to address metal contaminants. Treatment system results indicated that standardized treatment measures are required. The use of our water quality assessment toolbox provided more comprehensive detection and analysis of waterborne threats to the public. These data can help local governments and non-governmental organizations to select appropriate treatment solutions.

1265

INTEGRATION OF A SAFE WATER SYSTEM WITH ANTENATAL SERVICES, MACHINGA DISTRICT, MALAWI, 2010-2011

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Antenatal clinic (ANC) visits provide an opportunity to integrate additional interventions to improve maternal and neonatal health and motivate pregnant women to attend ANC services. In Malawi, although 93% of women attend at least one ANC visit, 57% deliver in health facilities, and 7% have postnatal checks. To reduce the risk of diarrhea, a leading cause of childhood mortality, we integrated free hygiene kits (safe water storage containers, water treatment solution [*WaterGuard*], soap, and oral rehydration salts) with ANC services. To receive the hygiene kit, women had to have a spouse/partner present; HIV testing was also offered to the couple. At subsequent ANC visits, up to 3 refills of *WaterGuard* and soap were provided. We surveyed 106 women receiving ANC care at baseline before program implementation and at follow-up 12 months later to assess water treatment; test drinking water for residual chlorine; observe hand-washing; and determine ANC service utilization. From baseline to follow-up, there was an increase in the percentage of women who had ever used *WaterGuard* (38% vs. 100%, $p<0.001$), knew how to use it correctly (23% vs. 81%, $p<0.001$), were observed to have a bottle in their home (3% vs. 77%, $p<0.001$), had residual chlorine in their stored water (0 vs. 71%, $p<0.001$), and were able to demonstrate proper handwashing technique (21% vs. 65% $p<0.001$). At follow-up, 89% of respondents had ≥3 ANC visits, 90% delivered at a health facility, 99% were tested for HIV, 99% of partners were tested for HIV, and 98% had disclosed their status to their partner. Women in this program showed statistically significant increases in water treatment and hygiene practices, and high utilization of ANC services and HIV testing. This evaluation suggests that integration of hygiene kits, refills, and HIV testing during ANC is feasible, can serve as an incentive to increase use of health services, and may help motivate changes in health behavior.

1266

IMPACT OF COMPLEXITY OF HANDWASHING INSTRUCTIONS ON ADHERENCE IN A LOW INCOME SETTING, DHAKA, BANGLADESH, 2010

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Handwashing reduces diarrhea risk in young children. Interventions to improve handwashing usually include instructions on how and when to wash hands. These instructions vary in complexity, with some recommending multiple steps including duration of lathering and scrubbing various aspects of the hands. To assess whether complex handwashing instructions result in reduced adherence, we conducted a randomized trial in a low-income area of Dhaka, Bangladesh. Mothers of young children were randomly assigned to one of three sets of handwashing instructions: simple, moderate, or complex. Simple instructions were to wet, lather, and rinse hands; moderate instructions included simple instructions and additional steps to scrub palms, scrub backs, and dry hands by waving them in the air; complex instructions included moderate instructions and additional steps to scrub between fingers, scrub under nails, and lather for 20 seconds. The field worker

taught the participant the randomly assigned set of instructions, without mention of the other two sets. Immediately, two days, and two weeks after the teaching, participants were asked to demonstrate handwashing to the field worker. Adherence was defined as demonstration of all of the instruction steps prescribed for the assigned treatment arm. We enrolled 244 participants (simple n=85, moderate n=75, complex n=84). Compared with the simple group, in which 100% adhered to prescribed instructions at all post-intervention assessments, the more complex groups had lower adherence at two weeks (moderate 43%, p<.0001; complex 31%, p<.0001). Adherence to air-drying hands was low at immediate, Day 2 and Week 2 assessments (moderate: 49%, 39%, and 47%; complex: 57%, 46%, and 38%). Exclusion of the air drying step from the outcome yielded adherence rates of 99%, 91% and 88% for the moderate group and 81%, 69% and 71% for the complex group. In a low-income community in Dhaka, highly complex instructions for handwashing resulted in decreased adherence. Future research should investigate whether adherence to the highly complex set results in greater hand decontamination than adherence to the simple or moderate set of instructions. When developing materials to promote handwashing behavior, handwashing promotion programs should consider the complexity of the overall set of instructions, as well as the microbiological impact and feasibility of adherence to specific instructions, such as air drying.

1267

CONSISTENT SOAP AVAILABILITY CORRELATES WITH USE OF INEXPENSIVE SOAP PRODUCTS AND IMPROVED HANDWASHING BEHAVIOR IN LOW-INCOME HOUSEHOLDS IN DHAKA, BANGLADESH

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Handwashing (HW) with soap reduces diarrhea in children < 5 years of age in low-income countries. Understanding characteristics of households with increased HW could inform interventions to increase this behavior. Amongst low-income households in Dhaka, Bangladesh, we studied consistent soap availability as a possible indicator of increased HW. Households were selected randomly from controls of a case-control study on hygiene and respiratory illness; all had ≥ 1 child ≤ 5 yrs. We visited 220 households 8 times over 4 weeks in Feb-Mar 2010. Respondents were interviewed about soap availability. Fieldworkers observed the presence of soap and water at HW stations, the cleanliness of respondents' palms and administered a validated 14-question tool on the strength of handwashing habits. We used data from structured observations, conducted several months prior, to estimate HW behavior at critical times. We used logistic regression to adjust for socioeconomic status, and compared households that had soap for HW available at 100% of visits to households that did not. Soap for HW was available in 1513 (88%) of 1716 visits to 220 households. In 110 households (50%), soap was available at every visit. Compared to those with inconsistent soap availability, households with consistent soap availability at each visit were more likely to be in the highest socio-economic status quintile (determined by principal component analysis on household assets) (OR 1.9; 95% CI 1.4- 2.4), more likely to have soap present at the HW station (OR_{adj} 1.6; 95% CI 1.3- 2.0), more likely to wash hands with soap at critical times (OR_{adj} 1.4; 95% CI 1.1 -1.7) and more likely to identify cheaper detergent soap rather than bar soap as the main HW product (OR_{adj}2.2; 95% CI 1.6- 2.9). Consistent availability of soap for handwashing was not associated with scores for the key components of habit. Households that had soap available in the home during each visit were also more likely to keep soap where it was needed for washing hands, and to wash hands more frequently at times relevant for hand contamination and pathogen transmission. Reliance on less expensive soap may facilitate consistent soap availability, underscoring the importance of promoting affordable means of increasing handwashing. Interventions that emphasize soap availability and the efficacy of inexpensive soap may be effective in increasing handwashing behavior at critical times.

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IMPACT OF INTENSIVE HANDWASHING PROMOTION ON HOUSEHOLD TRANSMISSION OF INFLUENZA IN A LOW INCOME SETTING: PRELIMINARY RESULTS OF A RANDOMIZED CONTROLLED CLINICAL TRIAL

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Although handwashing with soap decreases the risk of all-cause respiratory illness, there is little published empirical evidence for the efficacy of handwashing with soap for prevention of influenza transmission in resource-poor settings. We tested the impact of handwashing promotion on the risk of household transmission of influenza, influenza-like-illness (ILI), and fever in rural Bangladesh. In 2009 and 2010, we identified index case patients (ICPs), individuals who developed ILI within the previous two days and were the only symptomatic person in their household. ILI was defined as fever in children <5 years old and fever with cough or sore throat in persons > 5 years old. Households were randomized to intervention or control). The intervention group received handwashing stations with soap and daily handwashing motivation at critical times for pathogen transmission, such as after coughing or sneezing. We conducted daily surveillance and tested household members with fever for influenza viruses by polymerase chain reaction. Secondary attack ratios (SAR) were calculated for influenza, ILI, and fever in each arm. We used logistic regression with generalized estimating equations to estimate the significance of the SAR comparison while controlling for clustering by household. Among 274 ICPs enrolled, 33 (12%) had laboratory-confirmed influenza infections. The SARs for influenza among household contacts of ICPs with confirmed influenza virus infection were 7.5% in the control arm (10/133) and 11.0% in the intervention arm (11/100) (p = 0.362). The SAR for ILI among household contacts of all ICPs was 11.9% in the control arm (146/1,226) and 14.2% in the intervention arm (186/1,314) (p = 0.232). SARs for fever were 12.1% and 15.0%, respectively, in the control and intervention groups (p = 0.113). When an intensive handwashing intervention was initiated after illness onset in a household member, we found no protective effect against influenza virus infections. Handwashing behavior may not have changed rapidly enough to match the pace of influenza virus transmission between household members. Courtesy bias among intervention households, who received daily motivation as well as hardware to facilitate handwashing, may have led to greater reporting of respiratory symptoms. Future efforts should consider whether handwashing behavior can be changed quickly after illness onset in order to blunt household influenza transmission.

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CONSUMER INPUT TO DESIGN AND DEVELOPMENT OF A NOVEL HOUSEHOLD WATER TREATMENT DEVICE

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We collected consumer preference data in urban, periurban, and rural areas in India and Indonesia to use in design and development of a novel POU device for use in Asia. The end product incorporates a

drinking water disinfection medium (registered by USEPA-#72083-3, 2009). Consumer exposures ranged from 1-month in-home use of functional prototypes, to use-pattern questionnaires, and from household placement of life-size cut-outs of proposed designs to 3- dimensional models based on these designs. Householders showed a preference for gravity feed device configurations that: could accommodate ~ 10 L of source water; allow for collection of filtered, disinfected product water after no more than a few hours; ensured collection of clear, uncolored water with no detectable taste, taint, untoward mouth-feel or odor on immediate consumption or after storage; offered ease of use in cleaning of upper chamber filtration elements; ensured high convenience in secure replacement of the water treatment train (prefilter/filter/adsorption media/ disinfecting cartridge) after a useful life of no less than several months' daily use (i.e., > 1000L); required minimal assembly at start-up; provided for ready access to product water via a faucet/outlet with reliable, drip-free function; and (critically important) had a 'modern' and attractive appearance, enhancing the household working and living environment. From in-home observations we determined that: construction needed to be robust, include auto- shut-off at the end-of-life, plus a visual indicator of approaching termination, and include an option for 'dialing in' varying efficacy levels (up to US-EPA 6/4/3). HaloPure Waterbird emerged from this process, a gravity-feed purifier capable of 6/4/3 log reduction, auto shut-off at 1500L (± 20%), and leak-free cam-lock cartridge placement. Imperceptible halogen residual provides for continued protection of product water, in the device or upon transfer. Listening to the "voice of the consumer" can lead to enhanced product design aimed at household water treatment device development.

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USE AND ACCEPTABILITY OF A POINT-OF-USE WATER FILTRATION DEVICE IN HIV-1 INFECTED ART NAÏVE KENYAN ADULTS

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Among HIV-infected adults and children in Africa, diarrheal disease remains a major cause of morbidity and mortality. WHO recommendations suggest HIV-infected individuals should treat drinking water at the point-of-use. While simple and effective water filtration devices are available, limited data exist regarding the use and acceptance of these devices in this population. We enrolled ART naïve HIV-positive adults into a two-year cohort study in western Kenya. Individuals were visited in their home at least once to assess acceptability and use of a study water filtration device. Of 417 participants enrolled and subsequently visited, most were female (81%), married (64%), had at least a primary school education (72%), and had CD4 cell counts above 350 cells/µl (76%). At enrollment, participants reported the most common sources of drinking water to be shared pipe or tank source (45%) followed by well water (25%) and river or stream (25%). Among participants with a functioning device, more than half (57%) reported using the water filtration device in all of the last 5 instances of obtaining water to drink (always) and 25% reported using the device at least 3 of the last 5 times. Only 3% reported never using the device. We found household monthly income greater than 5,000 Kenyan Shillings (~$57 US) to be associated with always using the device (OR: 2.12 (95%CI 1.23, 3.65)). A trend towards an association between increasing age and always using the device was also observed (OR per 5 year increase: 1.11 (95%CI 0.98, 1.30)). While 38% of participants reported drinking water outside of the home within the last 24 hours, most (77%) reported filtering their drinking water. Almost all participants found the device very acceptable, with 97% willing to purchase the water filtration device if their current device were to break. Providing simple point-of-use water filtration devices to HIV infected adults may be an inexpensive and practical intervention to improve water quality and reduce the risk of diarrheal disease among this high-risk population.

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EFFICACY VERSUS EFFECTIVENESS OF WATER CHLORINATION IN RURAL COASTAL ECUADOR

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Chlorination can provide a low-cost method of treating drinking waters and is known to be efficacious for reducing bacterial loads, but actual effectiveness under household conditions may not reduce microbial contamination to the same extent as under lab conditions. In a previous study we found no significant differences in log reductions in drinking water of households that reported chlorination of their water in rural coastal Ecuador. We present the results of a follow-up study at the same field site in which we observed and quantified chlorination procedures at the household instead of relying on self-reported chlorination. We also tested source waters and water from control containers stored under protected conditions outside of the household. We collected three sets of samples (source water, water stored in the home, and water stored under control conditions) from 145 households: 67 that did not chlorinate, 42 that used locally available chlorine according to local practices, and 35 that used chlorine dosed to recommended standards. Covariates included physicochemical data and household level indicators. The efficacy of chlorine treatment in our field laboratory-matched control samples was higher than the effectiveness in corresponding household samples, which is most likely the result of recontamination in the household during storage. Recontamination of water in containers in the household over a 24-hour storage period was observed between pairs of household and matched control samples for both E. coli and total coliform concentrations, with mean log differences ranging from 0.4017-0.6147 ($p < 0.0001$). 63.8% of samples had greater microbial contamination in household samples than in their matched control. The reduced effectiveness can also be explained by other factors such as source water turbidity, socio-economic status, unsafe water storage behaviors. Negligible disparities were found between the two chlorine treatment groups, suggesting that dosing practices did not greatly modify the relationship between chlorination and log reduction in contamination. Household effectiveness of chlorine treatment was significantly reduced over laboratory efficacy. This research provides important new insight about the relationship between household storage practices and chlorination under village conditions.

1272

ROTAVIRUS OUTBREAK AMONG CHILDREN IN DAY CARE CENTER, ZAPORIZHZHYA, UKRAINE

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In the city of Zaporizhzhya the incidence of acute gastroenterocolitis (GI) has recently increased. The proportion GI illness due to rotavirus infection (RI) has increased from 21.6% in 2008 to 40.6% in 2009. Among children incidence of RI has increased 2.5 times. We investigated an outbreak of GI in a daycare center (DCC). Samples of drinking water, food and human specimens were examined bacteriologically for intestinal pathogens. Ill persons, contacts and water were tested for rotavirus antigen by ELISA and dipstick testing. During a two week period in April, 17 cases of RI were reported. Cases were identified in 8 of 11 classes. In a class which attended only three hours per day there were no cases. Through testing we identified 11 carriers (1 caregiver from class 1 and 10 children). The highest incidence of RI (4 patients and 7 carriers) was observed in class 1 which consists of children under 3 years. The primary case was identified in this class. Rotavirus was found in these 4 children. Due to a staffing shortage care-givers served food to the children against normal sanitary

regulations. Using a retrospective cohort study design we established that the route of transmission was beet salad (RR=3,5; CI 1,07-11,36). The salad was served from a single bowl and distributed to children by class. Children from the two classes in which there were no cases of RI received the salad first. The first 4 cases of RI were not identified until laboratory testing was performed. The study established that the caregiver was infected at DCC. Transmission is believed to have occurred via asymptomatic carriers. Caregivers serving food to the other classes are thought to have transmitted disease to them. No cases of RI occurred in classes that received salad before class 1 was served. This study demonstrates the necessity of strict adherence to the sanitary and hygiene regulation in DCC's and the ongoing problem of RI.

1273

SPATIO-TEMPORAL PATTERNS OF DIARRHEAL DISEASE CAN REVEAL TRANSMISSION PATHWAYS IN AN EMERGING URBAN REGION OF ECUADOR

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Diarrheal disease is caused by a variety of pathogens that exploit multiple transmission pathways. The patterns of diarrheal disease in space and time may reveal which transmission pathways are dominant; e.g., direct person-to-person spread produces temporary clusters of cases; whereas environmental pathways result in constant clusters around environmental sources. We explored these spatial and temporal distributions of diarrhea in Borbón, a small urban region of northwestern Ecuador. The relationship between these patterns and household and neighborhood WASH characteristics was also estimated. We conducted a series of six nested case control studies between December 2008 and May 2009. Surveys were carried out monthly to collect data on WASH factors. The river as well as all houses and outdoor latrines were mapped using GPS. We employed spatial point pattern analyses assuming an inhomogeneous Poisson process. We used the K-function to measure clustering and the ratio of intensity between cases and controls to estimate spatial variation of risk by month. Generalized linear and generalized additive models were used to estimate the association between WASH factors and household diarrhea. We found both spatial and temporal variation of diarrhea in Borbón. The spatial variation was associated with different risk factors each month; the exception to this finding was living with children under five, which was found to be a consistent risk factor. For example, early in the rainy season (December and January), use of an unimproved sanitation facility was significantly associated with diarrhea. In the middle of the season, significant WASH effects were absent. Towards the end of the rainy season (May) better household hygiene was significantly protective for diarrhea. These results provide insight on where and when improvements to WASH factors may protect from diarrheal disease, highlighting the importance of indirect transmission through contaminated latrines in the dry season.

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RAPID VIABLE DETECTION OF HUMAN-ORIGINATED FECAL CONTAMINATION USING IMS/ATP AND QPCR TARGETING *BACTEROIDES FRAGILIS*

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Human-originated fecal contamination of our drinking water source and recreational water is a continuous public health concern around the world. Timely and cost-effective ways in detecting contaminants in water is very important for protecting human exposure to possible presence of potential enteric infectious agents. This study aimed to determine the effectiveness of a rapid detection method, immunomagnetic separation coupled with ATP bioluminescence (IMS/ATP) and qPCR targeting *Bacteroides fragilis* for human-specific contamination. *B. fragilis* is a strict anaerobic bacteria and is known to be one of the predominant microbial flora in human gut. For this, an on-site wastewater treatment system was used as a testing ground. Water samples were collected from various points: septic tank effluents; after bioreactor; and after chlorine dioxide treatment. The level of *B. fragilis* were tested with IMS/ATP using *B. fragilis*-specific antibody attached magnetic beads and qPCR targeting *gyr* B gene. The *B.fragilis* (Bf) levels measured by IMS/ATP showed 1.5 log reductions after bioreactor, and 2.0 reductions after ClO_2 treatment, respectively, when compared with the original levels in the septic tank. The Bf levels determined by qPCR showed 1.6 log reduction after bioreactor and 2.3 log reduction after ClO_2 treatment. The Bf levels measured by IMS/ATP and qPCR correlated well (y=0.8834x+0.8791, R = 0.998). In summary, IMS/ATP rapidly determined the levels of Bf in an on-site wastewater treatment system with sensitivity and specificity. Thus, it can provide near real-time (1.5 hr) results of the on-site wastewater treatment efficiency prior to its release into the environment. This is the first study that the new IMS/ATP assay targeting Bf was applied for determining on-site wastewater treatment efficiency. This assay can be applied for a broad range of rapid detection of human-specific fecal contamination in water where fecal contamination is suspected.

1275

FURTHER INSIGHTS INTO THE PHYSIOLOGICAL MECHANISMS THAT UNDERLIE TSETSE'S BENEFICIAL SYMBIOSES

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Bacterial symbioses are ubiquitous in nature, yet to date few studies have been performed to determine the physiological mechanisms that underlie these relationships. Insects represent a group of advanced multi-cellular organisms that harbor well-documented symbiotic associations. One such insect, the tsetse fly (*Glossina* spp.), harbors 2 maternally-transmitted bacterial symbionts, mutualistic *Wigglesworthia* and commensal *Sodalis*, that are intimately involved in maintaining the overall fitness of their host. In this study we examine the functional mechanisms that underlie these symbioses by producing tsetse flies that lack all of their endogenous microbiota. The resulting aposymbiotic offspring are highly susceptible to infection with normally non-pathogenic *E. coli*, and this immuno-compromised phenotype is characterized by the absence of phagocytic hemocytes and the irregular expression of immunity-related genes. When hemocytes collected from wild-type tsetse are transplanted into aposymbiotic flies, the recipient individuals regain their refractory phenotype. We also supplement the diet of pregnant aposymbiotic females with *Wigglesworthia* and *Sodalis* in an attempt to compliment the fitness of their offspring. Our observations provide further insights into the evolutionary adaptations that anchor the steadfast relationship shared between tsetse and its symbiotic microbes.

1276

PUNIQUE VIRUS, A NOVEL PHLEBOVIRUS, RELATED TO SANDFLY FEVER NAPLES VIRUS, ISOLATED FROM SANDFLIES COLLECTED IN TUNISIA AND ITS POTENTIAL IMPACT ON PUBLIC HEALTH

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Sand flies are widely distributed around the Mediterranean. Therefore, human populations in this area are exposed to sandfly-transmitted diseases, including those caused by phleboviruses. While there is substantial data in countries located in the northern part of the

Mediterranean basin, few data are available for North Africa. Sand flies were collected from the site of Utique, a well-known site of visceral leishmaniasis in northern Tunisia, during the summers of 2008, 2009 and 2010. In 2008 and 2009 sand flies were captured and pooled by sex and species. A vast majority of sand flies belong to *Phlebotomus perniciosus*. Thus species identification was abandoned in 2010 and sand flies were pooled by sex. Sand flies were tested for the presence of phleboviruses by PCR. Viral RNA corresponding to a novel virus closely related to Sandfly fever Naples virus (SFNV) was detected in pools of sand flies collected in 2008 and 2009. Virus isolation in Vero cells was achieved. Genetic and phylogenetic characterisation based on sequences in the three genomic segments showed that it was a novel virus distinct from other recognized members of the species. This novel virus was provisionally named Punique virus. Viral sequences in the polymerase gene corresponding to another phlebovirus closely related to but distinct from Sandfly fever Sicilian virus (SFSV) were obtained from positive pools collected in 2008 and 2010. Isolation of this virus temporarily named Utique Virus remained to be achieved. The public health impact of those two new viruses remained to be determined.

1277

ANTIBODY RESPONSES OF GUINEA PIGS TO SALIVARY ANTIGENS OF *TRIATOMA INFESTANS* FOR THE DEVELOPMENT OF TRIATOMINE EXPOSURE MARKERS

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Antibody responses to salivary antigens of the most effective vector of Chagas disease, *Triatoma infestans*, offer the potential to develop exposure markers for detecting the presence of small numbers of triatomines, especially after vector control measures have been implemented. Previous studies have detected a salivary apyrase as a main candidate exposure marker using guinea pig sera, but this protein is frequently found in the saliva of different haemathophagous insects and thus not triatomine specific. Futhermore, antibody responses to saliva of different developmental stages were not considered, although the immune responses may vary if using nymphal or adult saliva. Therefore in this study, guinea pigs were exposed weekly to 5 nymphs or adults of different *T. infestans* strains from Chile, Argentina and Bolivia over a period of 11 weeks and they were bled 5 days after each exposure. IgG responses of guinea pigs to nymphal and adult saliva were detected 11 days after the first exposure and both responses differed significantly. Saliva of nymphs and adults revealed complex protein profiles that uncovered differences not only between the *T. infestans* strains but also between the developmental stages. The most prominent bands in all strains were of 85, 72, 44 and 25 kDa. Although the saliva of nymphs was richer in its protein composition than the adult saliva, more salivary proteins of adults (n=10) were recognized by guinea pig sera than nymphal proteins (n=6) during the long-term study. Four antigens (85, 79, 72 and 44 kDa) were recognized by all guinea pig sera. Candidate exposure markers, such as a truncated pallidipin-like salivary protein (gi|148469123), were characterized, identified and synthesized as recombinant protein forms. The immunogenicity of these antigens was evaluated by sera of guinea pigs from the laboratory and field studies.

1278

FIELD EVALUATION OF A WICKING ASSAY FOR THE RAPID DETECTION OF RIFT VALLEY FEVER VIRAL ANTIGENS IN MOSQUITOES (*DIPTERA: CULICIDAE*)

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Rift Valley fever virus (RVFV) causes outbreaks of severe disease in domestic ungulates as well as humans in Africa. There is a concern that outbreaks of RVF may continue and that this virus may spread into regions where it had not previously been detected. Surveillance and rapid detection are critical to the initiation of an effective disease control program. Here we report on the field evaluation in Kenya of the VectorTest® RVFV antigen assay, modeled on the VecTest® assay for West Nile virus. The dipsticks provided results in less than 20 min, were easy to use, and did not require a laboratory with containment facilities. Although none of the field-collected mosquitoes were infected with RVFV, the dipstick provided a clear positive result with pools of field-collected mosquitoes spiked with a single positive, irradiated (to inactivate an infectious virus) mosquito. Similarly, the dipstick was able to detect virus from pools of mosquitoes captured during the RVFV outbreak in 2007. The RVFV dipstick assay was highly specific with only a single weak false positive out of 266 pools tested (specificity >99.6%). The RVFV assay can provide a rapid, safe, easy to use preliminary test to alert public health personnel to the presence of RVFV in mosquitoes in a given area. Results from this assay will allow for more rapid medical threat assessments and the focusing of vector control measures on high-risk areas.

1279

IDENTIFICATION OF A NEW GROUP OF LACTATION-ASSOCIATED PROTEINS IN THE TSETSE FLY, *GLOSSINA MORSITANS MORSITANS*

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Tsetse females generate a single larva during each gonotrophic cycle. All nutrients for larval development are provided by the mother in the form of lactation products generated by the milk gland. The nutrients within the milk are primarily composed of equal amount of lipids and proteins. Four proteins have been associated with tsetse lactation products, milk gland proteins 1-3 (*gmmmgp1-3*) and transferrin. However, little is known about other protein components of tsetse milk. In this study, we performed an Illumina based transcriptome analysis of differential gene expression in pregnant flies compared to those post parturition to identify lactation-specific genes. This analysis revealed 11 transcripts that are upregulated during pregnancy including the previously identified *gmmmgp1-3* and *transferrin*. Seven new MGPs (*gmmmgp 4-10*) were identified in this analysis. These proteins appear to be related as the amino acid composition of these proteins is similar to *gmmmg2-3*. Genomic analysis of *gmmmgp2-10* revealed that they are located on the same genomic loci. Analysis of the predicted upstream regulatory regions for *gmmmgp4-10* found conserved binding sites previously identified in the regulatory regions for *gmmmgp1-3*. Search for homologus sequences to these genes has only revealed a single uncharacterized sequence from the flesh fly, *Sarcophaga crassipalpis*. The predicted amino acid sequences for *gmmmgp2-10* contain a high percentage of hydrophobic amino acids and a conserved secretory signal peptide, however they lack characterized functional domains. Expression patterns of *gmmmgp2-10* are female specific and localized to the milk gland tissue. Temporal analysis of transcript levels for these genes is similar to the other genes

associated with lactation. This expression pattern results in increased transcript levels in correlation with larvigenesis followed by immediate decline after parturition (birth). Knockdown of *gmmmgp7* utilizing siRNA injections resulted in a significant reduction of fecundity. The discovery of *gmmmgp4-10* reveals a family of genes essential for viviparity and novel in form and function.

1280

RETENTION OF DUPLICATED LONG WAVELENGTH OPSIN GENES IN THE GENOMES OF THE MOSQUITO VECTORS *AEDES AEGYPTI, ANOPHELES GAMBIAE* AND *CULEX QUINQUEFASCIATUS*

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Understanding the role of mosquito vision in mating, host detection and oviposition, may help to improve or develop new control strategies to reduce the incidence of vector-borne diseases. Here we report the first molecular analysis of light receptors (opsins) from three mosquito vectors - the yellow fever mosquito, *Aedes aegypti*, the malaria mosquito, *Anopheles gambiae*, and the southern house mosquito, *Culex quinquefasciatus*. Opsins are receptors that interact with photons to initiate visual processes. Typically, insects have three classes of opsins that are stimulated by ultraviolet, short, and long (LW) wavelengths. We used expression data to improve the 10 *A. aegypti* and 11 *A. gambiae* published opsins gene models, and we report the first manual annotation of 13 opsin genes from *C. quinquefasciatus*. *Opsin* transcripts were confirmed using published expression data and Reverse Transcriptase-PCR. Phylogenetic analyses predicted six putative LW opsins in *A. aegypti*, six in *A. gambiae* and eight in *C. quinquefasciatus,* suggesting an expansion of these genes in the Culicidae relative to other insect taxa. Time of divergence suggests the mosquito LW opsins originated from several duplication events between 167 to 1 million years ago (MYA), and that 15 LW genes may have originated following a duplication event that occurred approximately 126 MYA. LW opsins share approximately 100% and 60% amino acid similarity within and between mosquito taxa, which raises intriguing questions regarding the retention of these genes in the three mosquito genomes. Seven amino acids were identified under positive selection in the N and C termini, and one in a third trans-membrane domain suggestive of opsin spectral tuning. Conserved nucleotide sequence in 6 out of 38 ortholog pairs and in 8 out of 14 paralog pairs of the non coding regions, up- and or down-stream, of the LW opsins is indicative of coordinated gene regulation. We discuss potential mechanisms, including positive selection and differential gene regulation, for the conservation of LW opsins in these mosquitoes.

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FIELD USER ACCEPTABILITY EVALUATION OF A NOVEL, SELF-SUPPORTING, LONG-LASTING INSECTICIDAL NET (LLIN)

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Insect bed nets provide protection against arthropod-borne disease pathogens such as malaria, dengue, and leishmaniasis. United States Army service members currently have a choice between two types of bed nets to use in field environments; however, both have various limitations that preclude effective long-term use by non-mobile forces. Therefore, the US Army was faced with a challenge to develop an improved bed net that does not have any of the limitations associated with these existing bed nets. The Walter Reed Army Institute of Research has partnered with Tritons Systems, Inc. to develop a novel, self-supporting, long lasting, insecticide-impregnated net (LLIN). The purpose of this study was to evaluate the new bed net in comparison with the existing Standard and Self-Supporting Low-Profile bed nets using an acceptability threshold of 70%. Upon completion of a large scale field training exercise in which these bed nets were used over the course of several nights, soldiers completed a self-administered survey answering questions about their ease of use, setup, dismantling, and comfort. Results of this acceptability study will be presented in the context of military force health protection.

1283

IMMUNOGENIC AND BIOCHEMICAL PROPERTIES OF IXOLARIS, A TICK SALIVARY TISSUE FACTOR PATHWAY INHIBITOR

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Ixolaris is a potent Tissue Factor inhibitor from tick saliva. It binds to Factor X(a) and the binary complex Ixolaris/FX(a) interacts with FVIIa/TF thus blocking the coagulation cascade. Ixolaris has been sucessfully tested as an antithrombotic in rats, and also displays anti-cancer properties in a glioblastoma model. Because Ixolaris displays therapeutic potential, understanding its immunogenic and biochemical properties is of interest. Here we demonstrate that ixolaris elutes as approximately 18 kDa protein according to gel-filtration chromatography. Light scattering plot and ultracentrifugation experiments also indicate that Ixolaris is a monomeric protein of approximately 18 Kda. Since the predicted mol mass for Ixolari is 15.5 kDa, the discrepancy is attributed to glycosilation. This contention has been confirmed by a smear observed by SDS-PAGE and mass spectrometry analysis of Ixolaris. Elisa also demonstrate that Ixolaris is non-immunogenic in rabbits and in mice. Taken together, these results provide further support for the potential therapeutic use of Ixolaris in a number of conditions with abnormal expression of Tissue Factor, including thrombosis, cancer, sepsis and malaria.

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MOLECULAR MECHANISMS OF *WOLBACHIA*-MEDIATED VIRAL INTERFERENCE

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Dengue is one of the most important arboviral diseases currently threatening human populations, with over 50 million cases in tropical and subtropical regions each year. No treatment or vaccine is currently available for dengue fever. Recently, the endosymbiotic bacterium *Wolbachia* has been proposed to be used as a tool to reduce mosquito vectorial capacity for dengue viruses through population replacement. Our previous studies showed *Wolbachia* alone can induce resistance to dengue virus in *Aedes aegypti*, which was associated with a boosted basal immunity in the *Wolbachia*-infected mosquito. To understand the molecular mechanisms underlying *Wolbachia*-mediated viral interference, we examined *Wolbachia*-induced physiological changes in mosquito by comparison of genome-wide transcriptome between *Wolbachia*-infected and -uninfected *Ae. aegypti*. Experiments were also conducted to compare full scale physiological responses of the two groups of mosquitoes to dengue virus infection. We found that the Toll signal pathway was prominently activated by *Wolbachia* in response to dengue virus infection. Interestingly, the genes related to redox stress response systems and mitochondria were strickly regulated by the *Wolbachia* in *Ae. aegypti*. Further studies were also conducted to investigate how the Toll signal pathway was activated by *Wolbachia* in *Ae. Aegypti.* As the effector genes of Toll signal pathway, the defensins and cecropins genes induced by *Wolbachia* were confirmed to play roles in control of dengue infection. Our studies provide evidence to support that *Wolbachia* induces resistance to dengue virus in *Ae. aegypti* through modulation of host immunity. We discuss the results in relation to develop *Wolbachia*-based control strategies for population replacement.

1285

THE INFLUENCE OF HABITAT ON THE GENETIC STRUCTURE OF *GLOSSINA FUSCIPES FUSCIPES* IN UGANDA AND IMPLICATIONS FOR VECTOR CONTROL

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Human and animal forms of African trypanosomiasis represent a burden to the public health and economy of many African countries. For effective trypanosomiasis management, controlling its vector, the tsetse fly (Diptera: *Glossinidae*), is necessary, but long-term success in vector control efforts requires a better understanding of tsetse dispersal and breeding ecology. We have collected genetic data over several years in Uganda for a major trypanosomiasis vector, *Glossina fuscipes fuscipes* (*G.f.f.*). This genetic information coupled with publicly available environmental data (climate, hydrology, land cover) was used to assess habitat selection and dispersal and breeding capacity of tsetse in Uganda. Connection networks between *G.f.f.* sampling localities were constructed and a modified inverse distance weighting method was used on these networks to interpolate a 'landscape' of genetic variation in Uganda. Genetic variation captured in this way was used with environmental data to carry out environmental niche modeling in Maxent v. 3.3.3. The inferred distribution of *G.f.f.* represents the flow of genetic information on the environmental substrate of Uganda. We used circuit theory methods implemented in the program Circuitscape v. 3.5.4 to model genetic connectivity of the environmental landscape in Uganda and estimate environmental resistance to dispersal between *G.f.f.* populations. The environmental 'friction' estimates were used to explore local genetic structuring of tsetse flies via spatially explicit principal components analysis (sPCA) with the 'adegenet' R package. Environmentally explicit modeling of gene flow provides information about the influence of the environment on genetic variation and connectedness. Environmental-genetic inferences about habitat selection and dispersal in tsetse could substantially improve vector control by helping to identify areas to be targeted for control and minimizing the probability of re-infestation from neighboring areas.

1286

FINE-SCALE GENETIC DIFFERENTIATION OF *GLOSSINA FUSCIPES FUSCIPES* IN THE LAKE VICTORIA BASIN AND IMPLICATIONS FOR VECTOR CONTROL

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The primary vector of Human African Trypanosomiasis (HAT) in Uganda is *Glossina fuscipes fuscipes* (G.f.f.). Little information is available on genetic differentiation and population dynamics of G.f.f. in the Lake Victoria basin. We screened for genetic diversity among tsetse populations both on mainland and island sites in southern Uganda. The aim of this work is to provide empirical data to support short-term vector control efforts and inform long-term monitoring with the ultimate goal of creating tsetse free zones. We used genetic data from 19 microsatellite loci and the mitochondrial cytochrome oxidase gene (530bp) to estimate population sizes and levels and patterns of genetic differentiation and gene flow within and among 13 tsetse populations in the Lake Victoria basin. We also used mark-release-recapture data to estimate population sizes and movement patterns and related these to genetic inferences. Temporal collections from the same sites were used to evaluate seasonal fluctuations (dry vs. wet) of tsetse demography. Both nuclear and mitochondrial markers suggest the existence of past and current genetic exchange among island populations and between island and mainland sites. We observed a positive correlation between geographic and genetic distance, which suggests that open water does not necessarily act as a barrier to tsetse dispersal. Genetic data also suggest that males disperse farther than females and that populations are stable over wet and dry seasonal cycles. We will discuss the results in light of other recent genetic studies and compare them to previous ones based on ecological data.

1287

THE CELL BIOLOGY OF *CANDIDATUS* RICKETTSIA ANDEANAE

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Candidatus Rickettsiae andeanae is an incompletely characterized spotted fever group rickettsia (SFGR), first detected in *Amblyomma maculatum* and *Ixodes boliviensis* ticks collected in 2002 from northern Peru during a febrile outbreak investigation. Phylogenetic analysis of the 17-kDA, *gltA, ompB, ompA,* and *sca4* genes demonstrated alignment with SFGR, but the molecular isolates were not found to be identical to any rickettsial agent listed in GenBank, and *Candidatus* R. andeanae was deemed a novel rickettsial agent. Despite molecular characterization of the *Candidatus* R. andeanae, the successful *in vitro* cultivation of this bacterium has remained a challenge. We recently used one half of the *Candidatus* R. andeanae-positive *A. maculatum* tick collected in Portsmouth, VA to successfully infect cultures of Vero, DH82, and S2 cells. Infections were confirmed using quantitative real-time PCR (qPCR) assays, acridine orange staining, and DNA sequencing of *gltA, ompB,* and *sca4* fragments. Current investigation of the cell biology by electron microscopy of *Candidatus* R. andeanae shows that the coccobacillus is approximately 0.3 um long and 0.2 um wide, it has a double cell membrane similar to other SFGR, but it is has only been observed growing in the cytoplasm and not in the nucleus of the three cell lines assessed. Nuclear extraction studies are ongoing to more specifically determine if this agent replicates within the nucleus. The studies described herein will more fully characterize this newly discovered rickettsia, which has now been established in culture for the first time in our laboratory.

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THE ROLE OF BIOFILM FORMATION IN COLONIZATION AND TRANSMISSION OF THE COMMENSAL SYMBIONT *SODALIS GLOSSINIDIUS* WITHIN THE TSETSE FLY

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Awareness of diversity and abundance of beneficial microbes has greatly increased with the advancement of molecular technologies. Recently, the influence of beneficial microbes in onset or prevention of disease has been shown, indicating an opportunity for harnessing these microbes for control of disease. *Sodalis glossinidius* is a gram-negative commensal symbiont of the tsetse fly, the sole vector of the African trypanosome. *Sodalis* is harbored throughout the fly both intra- and extracellularly, primarily in the midgut tissue in close proximity of the trypanosome and is maternally-transmitted to offspring. The proximity and the ability to genetically manipulate *Sodalis* makes it a great candidate for paratransgenesis; i.e., expression of antitrypanosomal compounds in the *Sodalis* within tsetse's midgut. One essential aspect of paratransgenesis is understanding transmission biology of *Sodalis* and recolonization efficiency of genetically modified *Sodalis* in tsetse lines. Biofilms are dense populations of microbes that adhere to surfaces and each other secreting extracellular polymers. Only a few studies have shown the role of biofilm formation in vector born disease; i.e., *Yersinia pestis* within the flea gut. The ability of *Sodalis* to produce a biofilm was investigated using a classical microtiter plate biofilm assay and was shown to produce a biofilm under *in vitro* conditions. In this study we assessed genes important for biofilm formation in the fly gut

colonization process, transmission to progeny and trypanosome infection rates. Our studies provide enhancement of paratransgenic methodology by understanding the role of biofilm formation in both recolonization of *Sodalis* and trypanosome infections, which will guide us in applying paratransgenesis in the future.

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NEW VECTOR CONTROL MATERIALS FROM THE ARMED FORCES PEST MANAGEMENT BOARD

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The Deployed War-Fighter Protection research program (DWFP) is an initiative to develop and validate novel methods to protect United States military deployed abroad from threats posed by disease-carrying insects. Starting in 2004 and administered by the Armed Forces Pest Management Board the program is funded at $5M per year. The DWFP research portfolio is concentrated in 3 specific areas: novel insecticide chemistries/formulations, application technology, and personal protective systems. Program consists of a noncompetitive funding process for USDA ARS-based research, and a competitive grants process open to non-USDA ARS scientists (PIs from academia, industry, and military entomologists: 55 projects funded). Up to $3 million per year is given to USDA ARS National Program 104, dealing with Veterinary, Medical, and Urban Entomology. Ultimate objective is to find industry partners and get useful products into the market/military stock system. Presentation highlights DWFP products with examples of equipment, insecticides, and ~300 refereed publications.

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COMPREHENSIVE EPIDEMIOLOGICAL RESEARCH EFFORT ON FEBRILE ILLNESSES AND HEMOGLOBINOPATHIES ALONG THE BANGLADESH-MYANMAR BORDER

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In order to estimate the burden of febrile illnesses in the border region of Bangladesh toward Myanmar a comprehensive prevalence study on febrile illnesses and hemoglobinopathies was conducted in an area with suspected high endemicity of tropical infectious diseases. Little is known about the prevalence of febrile illnesses in the Chittagong Hill Tracts, the southernmost region of Bangladesh bordering Maynmar and India, an area with limited access to medical care due to inaccessible terrain and lack of infrastructure. Samples were collected from patients enrolled during two separate cross-sectional studies in the years 2007 to 2010 covering the same rural communities in rainy and dry season to assess seasonal trends. In a parallel ongoing hospital-based fever survey data of febrile participants from the catchment area of the Bandarban Sadar Hospital were collected. Out of a total population of 2123 enrolled in the studies 671 acute febrile patients were diagnosed for the most common infectious diseases: malaria (RDT, microscopy and PCR), typhoid fever, leptosirosis, dengue (serological assays) and influenza (RDT and PCR) as well as hemoglobinopathies. The collected data allow for an estimation of long term trends in the epidemiology of the investigated diseases as well as short-term variations such as seasonal fluctuations and emergence of rare conditions. *Falciparum* malaria remains the major health threat with a cross-sectional prevalence of 40.9% (CI: 35.4 - 46.7%) during monsoon months (May - October). However numbers vary significantly with the season and show an overall declining trend over the years. A high number of seropositive cases for leptospirosis (n= 194, 28.9%; SD: 25.6 - 32.5%) and typhoid fever (n=203, 30.3%; SD: 26.9 - 33.8%) indicate a major persistent reservoir of infection for these pathogens in the surveyed communities. Associations of disease distribution with demographic, geographic, and meteorological data were performed to define and map the prevalence and indirect estimates of incidence as the basis for assessing actual disease burden.

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INVESTIGATION OF A SUSPECTED OUTBREAK OF ACUTE FEBRILE ILLNESS IN MALINDI, KENYA IN DECEMBER 2010

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Acute febrile illness (AFI) refers to sudden illnesses with fever. It is a common clinical presentation in Kenya where its aetiology remains unknown. Information on the prevalence and causes of AFI in Kenya is limited. Walter Reed Project's (WRP) AFI surveillance site in Malindi noticed a 3 fold surge in AFI cases from Sept 2010 to Nov 2010 (Sept #10, Oct #5, Nov #15 cases). On average 2 cases are enrolled monthly. This prompted an investigation. Of note cases were from the same locality. Aliquots of malaria negative blood by RDT were sent to WRP reference lab for PCR and ELISA for Malaria, Salmonellosis, Brucellosis, Leptospirosis, Aborviruses and nasopharyngeal swabs tested for Flu. Study team: WRP and MOH. Study Area: Malindi District Hospital and Kisumu-dogo area. Investigation Period: 15 - 18 Dec. Case size: All 21 cases. A standard questionnaire was administered to all. All lab records from the period were reviewed. Data from the questionnaires was entered into an EPI-INFO database and analyzed. Majority, males (57.1%, n=12). The median age 30 yr. Most below 20 yr (42.9%, n=9). Most from Kisumundogo (23.8%, n=5). Majority presented with headache (42.9%, n=9), joint aches (28.6%, n=6) and myalgias (19%, n=4). 42.9% of cases classified as having AFI actually had undiagnosed malaria. 42.9% were malaria positive on PCR. All cases were negative for viruses by PCR, Elisas and cell culture. Importantly, the aetiology of fever remained unknown in 57.1% of cases. Malaria RDT's are not sensitive enough in low malaria transmission areas and when parasitemia is low. A negative RDT may not be enough to rule out malaria in regions of low malaria endemnisity. There is clinical and lab evidence of low parasitimia having being cleared in malaria immune patients as no PCR positive was given antimalarias but on repeat PCR all were negative for malaria. This could be explained by self treatment but all denied it. A comprehensive study to discover both common and uncommon pathogen causes of acute febrile illnesses is needed. PCR may be a complement to RDT and Microscopy in low malaria endemic areas. Continue vector control. Malaria naive persons should continue to be offered prophylaxis or preventive measures.

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EFFICACY, SAFETY AND PK OF ARTEMETHER-LUMEFANTRINE DISPERSIBLE TABLET IN THE TREATMENT OF ACUTE UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN INFANTS <5 KG BODY WEIGHT

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WHO recommends artemisinin-based combination therapy (ACT) as first-line therapy for infants with uncomplicated *Plasmodium falciparum* malaria who have body weight (BW) ≥5kg. However, no ACTs are indicated in infants <5kg. Poor safety profile of current standard of care,

quinine, limits its usage. Coartem (20mg artemether-120mg lumefantrine, AL), with an available pediatric formulation, has the largest clinical trial and postmarketing safety experience in infants ≥5kg to-date. This open-label, single-arm, multicenter study in Sub-Saharan Africa will enroll inpatient neonates and infants of <5kg BW with a confirmed diagnosis of uncomplicated *P. falciparum* malaria in two sequential cohorts of 15 infants each: term age >28 days (cohort 1) and term age ≤28 days (cohort 2) to minimize any theoretical risk. A joint data monitoring committee will review efficacy, safety, and pharmacokinetic (PK) data from cohort 1 and recommend whether to proceed to cohort 2, with or without dose adjustment. The primary objectives are to evaluate the efficacy and safety of AL dispersible tablet administered as 1 tablet bid over 3 days (to adjust if required), and to determine plasma levels of artemether, its active metabolite dihydroartemisinin, and lumefantrine. Exclusion criteria include severe malaria, signs and symptoms of a critical condition, hepatic or renal abnormality, and major neurological malformation. Neurodevelopment status follow-up of patients is planned until day 42 and at 3, 6 and 12 months. Primary endpoint is PCR-corrected parasitological cure at day 7. Secondary endpoints include reduction in parasite density at 24 hours; PK assessments; PCR-corrected parasitological cure at days 14, 28, and 42; time to parasite, fever and gametocyte clearance; and safety and tolerability assessments. Appropriate use of antipyretics and quinine as a rescue medication will be permitted. Protocol approval will be sought from ethics committees in Switzerland, and in each participating country. Written informed consent will be sought from all parents/guardians. Study results are expected in 2014.

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EVALUATING THE READINESS OF OUTPATIENT HEALTH FACILITIES TO MANAGE MALARIA CASES IN BENIN

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In 2008, the government of Benin and its partners began implementing a new national malaria case-management policy in 787 public health facilities (HFs) that recommended the use of artemisinin-based combination therapy (ACT). We evaluated the readiness of outpatient HFs to manage malaria cases in Benin about one year later. In late 2009, we conducted a nationally representative cross-sectional survey of a stratified random sample of 60 HFs. Surveyors observed consultations and interviewed and re-examined patients seeking care for any illness and pregnant women seeking antenatal care. In addition, health workers (HWs) were interviewed, and HFs were assessed to determine the availability of drugs and equipment. Results were weighted. Altogether, 57 HFs, 113 HWs, and 448 patients were included in the analysis. All HFs had a thermometer, 70.8% (95% confidence interval [CI]: 59.3-82.3%) had a scale for weighing children, and 66.3% (95% CI: 56.6-80.1%) of HFs had a booklet or chart with ACT algorithms. Although all hospitals could perform malaria testing, only 40.8% of non-hospitals could perform testing. In the three months before the survey, 46.7% (14/30) of hospitals and 33.3% (9/27) of health centers had stock-outs of all types of artemether-lumefantrine blister packs (i.e., none in stock) for at least three days. Adherence to the testing policy (i.e., test all patients with a febrile illness, and do not test patients without a febrile illness) was 52.9% (95% CI: 48.3-57.5%) among all 448 patients, 24.7% (95% CI: 18.2-31.2%) among 170 patients <5 years old, and 70.1% (95% CI: 64.7-75.5%) among 278 patients ≥5 years old. Nearly all of the 79 patients who tested positive were given an antimalarial (98.2%; 95% CI: 95.3-100%). However, 22.1% (17/77) of patients who tested negative were given an antimalarial. HF readiness varied. HWs prescribed antimalarials when tests were positive. However, ACT stock-outs were common, HWs did not follow testing guidelines for children <5 years old, and they sometimes prescribed antimalarials even when tests were negative.

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INVESTIGATION OF A CLUSTER OF DEATHS ATTRIBUTABLE TO MALARIA IN RURAL SENEGAL

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Since 2005, malaria control interventions, including insecticide-treated nets, rapid diagnostic tests, and artemisinin-combination therapies have been scaled up in Senegal, resulting in a large decrease in the malaria-associated morbidity and mortality. However, in Touba district, deaths attributed to malaria from September - November (transmission season) increased by 58% in 2009 compared to 2008, while admissions for malaria decreased 19%. One health center reported 80% (38/47) of the deaths. The National Malaria Control Program led an investigation of these deaths, consisting of interviews with families and care providers and retrospective chart reviews. Charts were reviewed for 38 malaria deaths, all confirmed by rapid diagnostic testing. The median age was 5 years (39% < 5 years and 37% 5-9 years) and 59% were male. Only 17% (6) sought care within 24 hours of symptom onset, with a median of 3 days. Anemia was laboratory-confirmed in 37% (14) and diagnosed clinically in 26% (10); mean hemoglobin was 3.9 g/dL in those tested, two of whom received a blood transfusion. Rapid blood glucose was performed in 18% (7) and complete blood counts in 37% (14). Of the 12 patients with elevated leukocytes, 8 received an antibiotic. Of 37 patients for whom treatment was documented, 65% received a correct medication regimen, 26% had dosing errors, and 5% died prior to starting quinine. The majority (92%) of patients died the day of admission or the following day. Recommendations included expanding the home-based management program, reinforcing preventive community based interventions, educating the community on danger signs and accessibility of treatment, and staff training to improve referral practices, performance and documentation of history and physical exams, complete blood counts and blood glucose, correct dosing of antimalarials, and differential diagnosis of fever. Additional studies would be needed to determine if the delays in seeking care and deficits in care were associated with the deaths. A standardized tool for investigation of deaths will help improve case management and the response to clusters of deaths.

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EFFECTS OF GASTROENTERITIS EPISODES ON MAINTENANCE OF POLIO VACCINE TITERS IN CHILDREN THREE YEARS AND UNDER IN RURAL COASTAL KENYA

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Evidence shows that infants with concurrent gastroenteritis (GE) are less likely to respond to oral polio vaccination than those without gastroenteritis. Our objective was to determine whether further episodes of gastroenteritis in the first three years of life had an effect on maintenance of polio titer. Children enrolled in a birth cohort in rural coastal Kenya received four trivalent polio vaccinations before 6 months of life. Sera were then drawn at 6 month intervals until age 36 months and polio titers were measured using poliovirus IgG ELISA kits. GE episodes were documented during scheduled follow-up visits and at any time of illness during the 3 year period. Student's t-test was performed to compare those with and without GE at each time point. Of 545 children in the study, 159 had 246 episodes of gastroenteritis in the first three years of life. GE episodes were more likely to occur between 6 and 18 months of life. The range of GE episodes per child was 0-4. Polio titers did not significantly differ between children with and without GE from 6 to 36

months of age. Although concurrent gastroenteritis may hamper immune response to oral polio vaccine, further episodes of gastroenteritis after the vaccination series do not appear to alter the maintenance of polio titers.

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CLINICAL IMPLICATIONS OF ADHERENCE TO WHO GUIDELINES FOR THE MANAGEMENT OF THE FEBRILE PHASE OF DENGUE

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According to WHO guidelines the use of acetaminophen is indicated during the febrile phase of dengue and aspirin or non-steroidal anti-inflammatory agents (NSAIDs) should be avoided as these drugs may aggravate gastritis or bleeding. However, there is little clinical evidence to support this recommendation. We conducted a prospective cohort study in an endemic area in Colombia to evaluate the potential association between noncompliance with this guideline and the risk of developing severe dengue. Acute febrile outpatients (less than 96 hours of onset) with dengue (confirmed by viral isolation, RT-PCR or a shift from negative to positive IgM test) were followed daily until the seventh day of disease. Subjects were excluded based on the following: diabetes, AIDS, hematologic disorders, cancer or cardiac disease and the presence of a major bleed, albumin < 3gr/dL, effusions or shock at presentation. Inappropriate Initial Treatment (IIT) was considered when the patient reported having taken NSAIDs, aspirin or dipyrone. Data collected included signs and symptoms, and daily microhematocrit determinations to recognize hemoconcentration. A complicated case was defined by the following: a platelet count ≤100.000/mm^3; any spontaneous hemorrhagic manifestation (or one positive tourniquet test); and evidence of plasma leakage (i.e. pleural effusion, ascitis, hypoalbuminemia or a variation of hematocrit greater than 10%). Of 596 patients, 97% appropriately received acetaminophen but 54% also received IIT. 63.2% (n=98) of cases receiving IIT were complicated compared with 36.8% (n=57) complicated cases in the 271 subjects treated only with acetaminophen [OR crude: 1.62 ; 95% CI: 1.96- 6.39; OR : 1.51; IC95% (1.03-2.2) adjusted by age and sex]. In conclusion, adherence to WHO guidelines during the febrile phase of dengue is important to reduce the risk of complications. This study is registered with Colciencias (Departamento Administrativo de Ciencia, Tecnología e Innovación de Colombia), number: 110245921561.

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MALARIA PREVALENCE AND MORTALITY IN RURAL SIERRA LEONE

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Malaria is a leading cause of morbidity and mortality in rural areas of Sierra Leone. Mortality from malaria is as high as 28% in the under age 5 group of pediatric patients. The Village Medical Project provides medical care and treatment to women and children in several villages in Gorama Chiefdom, Kono District of rural Sierra Leone The purpose of this study is to document the prevalence of malaria infection, anemia and crude mortality. The project has been working for 3 years to ascertain the success of primary treatment and prevention of malaria in a rural area of Sierra Leone. Adult and Pediatric Patients were tested for *Plasmodium falciparum* malaria and hemoglobin. Patients were selected from each village based on prior census data and followed for a 2 year period, from 2008-2010. Primary prevention with bed nets were provided for children under 5 years of age. 1043-1463 patients were seen annually over a 2 year period from 2008-2010. Overall, malaria prevalence varied from 67-97%. The overall crude mortality rate from 2008-2010 was 8%. Under age 5 mortality is 9.8%. 87% of the population is anemic based on WHO standards. Access to Medical Care and treatment remains difficult for this population We are significantly limited by lack of accurate ages, mobility of the population and changing demographics. Malaria is very prevalent in this rural area of Sierra Leone. Primary treatment and prevention has had some impact on mortality rates compared to prior studies, however there still remains a significant disease burden in this area, with significant morbidity and mortality.

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ARTEMETHER, DIHYDROARTEMISININ AND LUMEFANTRINE DO NOT INDUCE *IN VITRO* DRUG METABOLIZING ENZYMES AND METABOLISM OF ORAL CONTRACEPTIVES

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The goal of this study was to evaluate *in vitro* the components of Coartem/Riamet (artemether and lumefantrine) and the active metabolite dihydroartemisinin (DHA) for their potential to induce drug-metabolizing CYP enzymes and the metabolism of oral contraceptives. The experiments were conducted according to the FDA drug drug interaction guidance. The assessment was done *in vitro* in cryopreserved primary human hepatocytes of at least three individual donors. Induction of mRNA, relative to the vehicle control, was determined by real-time PCR and evaluation of changes in cytochrome P450 (CYP) enzyme activities were assessed after 48-h induction periods by LC/MS/MS analysis of CYP-selective probe substrate metabolism. Metabolism of the oral contraceptives was tested by HPLC analysis. Human hepatocytes were incubated with the three test substances up to concentrations which exceeded their therapeutic concentrations by a factor of 10. Ethinyl estradiol and levonorgestrel were selected as active ingredients of oral contraceptives and were tested at their therapeutic concentrations of 1 nM and 20 nM, respectively. Rifampicin at 0.1, 1, and 20 µM, and phenobarbital at 1000 µM were used as positive controls for induction of genes regulated by PXR and/or CAR like CYP2B6, CYP2C, and CYP3A; β-napthoflavone at 10 µM was included as positive control for AhR-mediated induction of genes like CYP1A. Artemether, DHA, and lumefantrine were determined not to be inducers of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, or CYP3A enzyme activity in hepatocytes or CYP1A1, CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP3A4, or CYP3A5 mRNA. Metabolism of ethinyl estradiol and levonorgestrel was determined not to be induced by artemether, DHA, and lumefantrine. As per FDA criteria, these conclusions were based upon the levels of mRNA or activity at least less than 2-fold, with respect to the vehicle control, and/or less than 40% of the maximal positive control induction response, indicative of a non-inducer *in vitro*.

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EFFECT OF DIARRHEA ON GROWTH IN INFANTS IN URBAN SLUM OF SOUTH INDIA

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Diarrheal diseases are the second leading cause of morbidity and mortality in children less than 5 years of age. The vicious cycle of diarrhea and malnutrition has long been recognized, and it has been shown that early childhood diarrhea has long term effects on growth and development. To study the association of diarrhea in early infancy with growth faltering at one year of age, longitudinal data (N = 897) from 3 birth cohort studies conducted between 2002-2011 in an urban slum of South India were analyzed. Children were followed biweekly/weekly to assess diarrhea and other morbidities. Monthly anthropometric (height/ weight) measurements were obtained. We assessed the effect of diarrhea, on acute (WHZ < - 2 SD) and chronic (HAZ < - 2 SD) malnutrition, using the WHO Child

Growth Standards 2006. The median number of diarrheal episodes among children in the cohort was 2 (1-3). At 1 yr, 33.9% of infants had chronic malnutrition and 26.9% had acute malnutrition. Three or more episodes of severe diarrhea was significantly associated with chronic (OR=2.45, *P*=0.02) and acute malnutrition (OR=2.8, *P*<0.01). Other factors associated with chronic malnutrition were living in a mud house, an indicator of lower socioeconomic status (OR=1.8, *P*<0.01), presence of an older sibling (OR=1.6, *P*<0.01). Duration of exclusive breasting, more than primary schooling as highest education in the family and being a girl offered protection of 22% (*P*<0.01), 44% (*P*<0.001), 30% (*P*<0.01) respectively. As expected, severity of diarrhea and poverty are associated with acute and chronic malnutrition, with exclusive breastfeeding and higher education being protective. Lower rates of malnutrition were noted in girls, an unexpected finding.

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U.S. MILITARY FORCE HEALTH PROTECTION POLICIES MAY IMPACT PEDIATRIC MALARIA PROPHYLAXIS PRESCRIBING PATTERNS

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To date, there have been no large scale systematic surveys of antimalarial prescribing practices in the United States. Although pediatric patients are at higher risk of severe disease due to malaria than adults, there is a relative scarcity of information on the prevention of malaria among pediatric travelers versus adult travelers. This study consists of a systematic search of the military health system electronic medical record system for all prescriptions of chloroquine (CQ), mefloquine (MQ), and atovaquone-proguanil (AP) to military family members 8 years of age and under in the years 2005-2010. Prescribing patterns were assessed for changes over time to identify if Department of the Army and Department of Defense policies, published in 2009, limiting the use of mefloquine in deployed forces coincided with changes in prescribing patterns for young children. A total of 3404 prescriptions were written for these medications during the study period. In total, CQ, AP, and MQ, respectively accounted for 7%, 43%, and 50% of all prescriptions. Overall prescription volume increased from a low of 507 prescriptions (60% MQ) in 2005 to a high of 726 (39% MQ) in 2010 (p <0.001). While the total volume of antimalarial prescriptions rose, this change was reflected almost entirely by an increase in the usage of AP. In 2010, in contrast to prior years, 44% of all AP prescriptions were for amounts in excess of a 30 day supply, compared to 37% for earlier prescriptions (p=0.015). This trend of progressively more prescriptions for AP in absolute and relative terms exists over the entire study period (p = 0.003). This study documents that military physicians providing pediatric travel services now prescribe less MQ relative to AP. This occurs even in settings where the duration of travel has led many experts to recommend MQ as the drug of choice. The timing of these changes suggests that military force health protection policy, as well as patient/family and provider awareness regarding adverse effects associated with MQ may be impacting prescribing practices for these medications.

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PRELIMINARY RESULTS OF A HOSPITAL-BASED LABORATORY SURVEILLANCE FOR INFECTIOUS ETIOLOGIES OF UNDIFFERENTIATED FEBRILE ILLNESSES IN GEORGIA

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Since 2008, laboratory-based sentinel surveillance for undifferentiated febrile illness (UFI) has been ongoing in six hospitals to establish the frequency of nine infectious causative agents of febrile illness in Georgia. Hospitalized patients ≥ 4 years of age with fever ≥38°C for ≥48 hours were asked to voluntarily participate. Blood culture and serologic testing (ELISA) were conducted for *Leptospira* spp., *Brucella* spp., West Nile virus (WNV), Crimean-Congo hemorrhagic fever (CCHF) virus, *Coxiella burnetii*, tick-borne encephalitis virus (TBEV), hantavirus, *Salmonella* Typhi and *Rickettsia typhi*. A total of 478 subjects have been enrolled in the study. Of these, 71% were outpatients and 53% were males with the mean age of 36 years. Fever of unknown origin was the preliminary diagnosis in 88% of patients. Patients also reported having fatigue (90%), rigors (87%), sweats (82%), pain in joints (48%), and sleep disturbances (40%). Acute and convalescent samples from 403 patients (n=473) were initially tested by IgM ELISA. Sixty-nine patients were seropositive for hantavirus (16%), 52 for *Leptospira* spp. (13%), 17 for *Coxiella burnetii* (4%), 16 for TBEV (4%), and 3 for WNV (0.7%). Additionally, 33 patients were seropositive for *Brucella* spp. (8%), 3 patients for *S.* Typhi (0.7%), and 8% (34) of patients showed positivity by IgG ELISA for *R. typhi*. Highest cross-reactivity was observed for hantavirus and *Coxiella burnetii,* in 13(2.8%) samples. Preliminary laboratory results indicate a high prevalence of antibodies against hantavirus, leptospirosis, brucellosis and rickettsioses among febrile patients in Georgia. This hospital surveillance for UFI has significantly enhanced laboratory capacity for the detection of specific infectious etiologies as well as established a valuable network of clinical sites that can be used for future syndromic surveillance studies. Confirmed laboratory results will allow the Georgian public health authorities to make better informed decisions regarding screening and prevention strategies.

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FACTORS INFLUENCING HIGH RATES OF CATCH UP GROWTH AFTER EARLY CHILDHOOD STUNTING IN CHILDREN OF URBAN SLUMS OF SOUTHERN INDIA: A COHORT STUDY

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Malnutrition and stunting in early childhood is a major public health problem in less developed countries. A lack of long term cohorts leads to a paucity of data on factors that influence catch up growth in children not enrolled in supplementary feeding programs. Our study in an urban slum in southern India investigated catch up in growth in children after early stunting. The study group was a birth cohort of 452 children,

followed intensively for three years, but at 7-8 years, 273 children were contacted in 2010. Data was collected using a structured questionnaire and anthropometry. For analysis, the cohort was divided into categories of children who were ever stunted at 12, 24 and 36 months and those who were never stunted. Of available children, 189/273 (69.2%) were ever stunted, but more than 80% of the 189 showed catch up growth by 2010. The mothers of the ever stunted group were younger by 1.4 years ($p = 0.009$), shorter ($p = 0.009$) and weighed less ($p = 0.02$) than mothers of never stunted children. Another variable that predisposed to stunting was household debt (Crude OR 1.82, 95 % CI 1.07-3.08). Ever stunted children were divided into 2 groups, persistently stunted (33, 17.5%) and children with catch up growth (156, 82.3%) at the current follow up. In univariate analyses, factors associated with catch up growth were having <3 children, use of sunflower oil, use of a ration card, schooling of child in an unaided private school and using liquefied petroleum gas as cooking fuel. After multivariate logistic regression analysis, the factor independently associated with catch up growth was use of a ration card (Adjusted OR 3.16, 95% CI 1.01-9.76). Our study shows remarkably high rate of catch up growth, which was associated with use of a ration card issued by the public distribution system, indicating that there is potential for governmental interventions to decrease malnutrition in poor urban communities.

1303

INFECTIOUS ETIOLOGIES OF ACUTE MENINGITIS AND ENCEPHALITIS IN GEORGIA

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In Georgia, there are diverse etiologies of meningitis and encephalitis, including vaccine preventable agents such as mumps virus, varicella zoster virus (VZV), *Streptococcus pneumoniae*, *Neisseria meningitidis*, *Haemophilus influenzae* type B (Hib), and others viral agents (e.g. Epstein-Barr virus (EBV), tick-borne encephalitis virus (TBEV) and West Nile virus (WNV)). Prevalence information regarding these infections in Georgia is limited. In October 2010, a hospital-based surveillance study was initiated to determine the incidence of infectious etiologies of acute meningitis and encephalitis; enhance laboratory capacity for the diagnosis of central nervous system (CNS) infections; determine antimicrobial susceptibility profiles; and describe the risk factors and clinical presentations associated with etiologic agents of CNS infections. Patients with suspected meningitis and encephalitis were enrolled from three hospitals in Tbilisi. Cerebral spinal fluid (CSF) and acute and convalescent sera were collected for bacterial culture and RT-PCR testing for HSV types 1 and 2, mumps virus, enteroviruses, VZV, *S. pneumoniae*, Hib, and *N. meningitidis.* The diagnosis of WNV, TBEV, and EBV was conducted via commercial ELISA assays. As of 21 March 2011, 66 patients have been enrolled (23 adults and 43 children) and 61 CSF samples tested. Initial laboratory results indicate the frequency of HSV-1, enteroviruses and VZV to be 43%, 38% and 2%, respectively. For both TBEV and WNV, the frequency was determined to be 7%. Nine samples were positive for TBEV and seven samples were positive for EBV in 131 pairs of acute and convalescent sera. One *S. pneumoniae* case was cultured from CSF. These preliminary study results suggest the presence of a wide-spectrum of pathogens among patients with suspected meningitis and encephalitis. This surveillance study serves as a model for enhancing patient care through understanding disease prevalence, building laboratory diagnostic capacity and designing future syndromic surveillance projects in Georgia.

1304

CLINICAL MANAGEMENT OF DENGUE: A PHYSICIAN EDUCATION PROGRAM TO IMPROVE CLINICAL OUTCOMES, PUERTO RICO

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In 2007-08, the Centers for Disease Control and Prevention (CDC) Dengue Branch and the Puerto Rico Department of Health (PRDH) conducted a survey to assess physician's knowledge of dengue and clinical management practices. The survey identified limited knowledge of warning signs for severe dengue and early signs of shock and non-standard treatment practices including use of corticosteroids and non-isotonic crystalloid solutions. A review of fatal dengue cases in 2007 corroborated these findings. In 2008-09, CDC Dengue Branch developed and pilot tested a physician training course to address the deficiencies identified by the survey and fatal case review. Focus groups and interviews were conducted with attendees of the pilot course to evaluate instructional process and content, and the course was revised accordingly. The course was approved by CDC and accredited by the Accreditation Council for Continuing Medical Education for 4 CME credits in February 2009. The Secretary of Health of Puerto Rico mandated that physicians take the training as a prerequisite for re-certification by 2013. To fully implement the case management course, 52 physicians were selected, trained and certified as Master Trainers. From February 21, 2009 to December 31, 2010, 55 courses attended by 8,301 of the 12,929 licensed physicians in Puerto Rico were conducted. Most physicians (6,294, 76%) were trained between September 1 and October 31, 2010 in response to another mandate from the Secretary of Health that all primary care physicians be trained immediately due to the increased number of dengue fatalities. An evaluation of the impact of training on clinical practices will be conducted in the Fall of 2011. Findings from this evaluation will be used to redirect continuing training efforts and to develop an online dengue clinical management course. Lessons learned from the implementation of this training initiative will be shared with dengue endemic countries planning to train physicians on the clinical management of dengue.

1305

PERFORMANCE OF STABILIZATION TUBES FOR EXTENDING TIME TO ANALYSIS OF COMPLETE BLOOD COUNTS FROM TRIAL PARTICIPANTS AT A RURAL FIELD SITE IN MALI

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Stabilization tubes (ST, Beckton Dickinson, Franklin Lakes, NJ) extend the pre-analytical stability of whole-blood (WB) specimens taken for CD4 measurements by utilizing a proprietary supplement targeted at

cell surface membranes and clusters of differentiation (CD) on white blood cells (WBC). We hypothesized that this membrane-protective effect might confer specimen stability by acting on other membrane-bound cellular components. ST were assessed using twenty (n=20) WB specimens collected during a malaria vaccine clinical trial in Mali, West Africa. WB specimens were collected into ST and EDTA Vacutainer tubes for comparison, and complete blood counts (CBC) were conducted at day 0 and then every 24-hrs for 7 days thereafter. All measurements of WBC parameters deteriorated (> 10% erroneous or missing values) after 24 hours post-collection, while all red blood cell (RBC) parameters remained largely unchanged through 6 days post-collection. Data analysis revealed that ST do not provide stability of WB after collection in our setting. Further investigations validating and implementing novel technologies in the field are greatly needed to ensure quality specimens are analyzed in clinical research.

1306

EVALUATING BLOOD CULTURES IN GUATEMALA AFTER IMPLEMENTATION OF A DEDICATED PHLEBOTOMY TEAM

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Blood cultures (BCs) are important diagnostic and surveillance tools to identify invasive bacterial diseases. In January 2008, we established automated BCs at a rural hospital in Guatemala and provided frequent trainings, job aids and all supplies and materials. After the first year of implementation, we found poor adherence to protocols and high contamination rates. In August 2009, we implemented a dedicated, round-the-clock phlebotomy team. To determine whether this intervention decreased contamination rates and improved pathogen isolation, we conducted segmented regression analysis of a monthly time series of BC outcomes from the laboratory database. A blood culture was defined as one or two blood culture bottles filled with blood taken from the same site. We collected data on 2,140 BC prior to intervention (January 2008-July 2009) and 1,525 blood cultures after the intervention was fully implemented (October 2009 to September 2010). There was an increase in the median number of BC per month among children <10 years old (41 per month pre-intervention vs. 58 per month post-intervention, p=0.05) but not among persons aged 10 years and older (63 per month pre-intervention vs. 68 post-intervention, p=0.69). Among 858 BC in children <10 years old during the pre-intervention period, 14% of cultures were contaminated and 7% produced a pathogen, compared to 10% contaminated and 4% with a pathogen among 695 cultures post-intervention. Among persons aged 10 years and older, 3% of 1282 cultures taken pre-intervention were contaminated and 8% yielded a pathogen, compared with 1% contaminated and 4% yielding a pathogen of 830 cultures taken post-intervention. Segmented regression analysis showed no impact of the intervention on the contamination rates among children <10 years old (β=-15.3, p=0.13) or persons aged 10 years and older (β=-2.5, p=0.45). Similarly, there was no effect of the intervention on pathogen isolation rates among children <10 years old (β=-2.2, p=0.62) or persons aged 10 years and older (β=-3.6, p=0.55). The results from this evaluation suggest that contamination rates among young children are unacceptably high and may be preventing isolation of pathogens. In addition to strengthening efforts to reduce contamination during venipuncture, particularly of young children, a review of laboratory protocols and procedures may identify further opportunities to reduce contamination and identify meaningful pathogens.

1307

"LOOKING FOR GOLD, FINDING MALARIA" THE 2010 MALARIA SURVEILLANCE OF THE SURINAME GOLD MINING MALARIA CONTROL PROGRAM

Hedley Cairo

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Malaria is endemic in the forested interior of Suriname. Since 2006 malaria cases have declined tremendously with dispersed foci remaining in the gold mining areas. Currently the majority of malaria infections occur among persons (ca. 15,000) engaged in small-scale gold mining and related activities. Because there were no formal health services in these remote areas, a Global Fund supported malaria program was initiated in 2009 to fill the gap. This control program builds further on a surveillance system established in 2006 as a pilot under the Medical Mission Malaria Program. The surveillance system gathers weekly information from the Tourtonne diagnostic and treatment facility in the Capital city Paramaribo and from a network of 18 home-based diagnostic and treatment facilities (Malaria Service Deliverers) in the gold mining areas. Malaria cases are diagnosed by blood film or rapid diagnostic tests. A descriptive analysis of preliminary surveillance data of 2010 will be presented and compared with data from the previous year. The system recorded 1548 cases of malaria in 2010 among gold miners; 1388 (90%) confirmed by microscopy and 160 RDT cases. This number represents a decrease of 31.5% from the 2259 cases reported for 2009. *Plasmodium falciparum* 39%, *P. vivax* 52%, mixed *P. falciparum* plus *P.vivax* (7%) and *P. malariae* (2%) were the species identified. Among the 1548 cases 961 (62%) were classified as imported from neighboring countries and 26 (2%) were of unknown origin. 40 cases were reported in pregnant women of which 8 were *P. vivax* relapse. The Annual Blood Examination Rate (ABER) was 52.83, Slide Positivity Rate (SPR) was 17.51 and the Annual Parasitic Index (API) calculated from autochthonous cases was 30 in 2010 compared to ABER 48.34, SPR 22.45 and API 39 in 2009. In comparison to 2009 a notable decrease in the number of malaria cases from gold mining areas was reported in 2010. Conveying the importance of adhering to appropriate preventive measures for malaria to the population at risk is mandatory for the decrease in malaria cases to be sustainable.

1308

ADULT REFERENCE VALUES FOR COMMONLY USED BIOCHEMICAL AND HEMATOLOGICAL TESTS IN CENTRAL GHANA

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Laboratory results and clinical examinations, provide useful information in screening, diagnosing and monitoring of diseases. Interpretation of laboratory results depend on reference values obtained from apparently healthy individuals from the population they are intended to serve. The reference values obtained from healthy residents of the communities used for clinical studies will help in determining eligibility and assessing the safety of those participating in these studies. This study was aimed at establishing gender-specific haematological and biochemical reference values for healthy adults in central Ghana. A total of 625 adults between the ages of 18 and 60 were enrolled within Kintampo and its environs. The medians, 2.5th and 97.5th percentiles were determined for five haematology and five biochemistry parameters commonly considered during screening/enrolment and follow up monitoring of individuals who usually participate in clinical trials and also for health management.

The Clinical Laboratory and Standards Institute (CLSI) guidelines for defining reference values were used. Values established in this study were compared with those derived in the developed countries. The percentage of our healthy population which had out of range values based on the data from the United States and the United Kingdom were determined. The red blood cell (RBC)) parameters (haemoglobin, haematocrit and RBC count), total leucocyte and platelet counts and urea values were significantly lower compared to values derived in the developed countries. Higher values were, however, obtained in our study for parameters such as Alanine aminotransferase, aspartate aminotransferase and total bilirubin. Up to 53% and 75% of the haematology and biochemistry values, respectively from our healthy population would have been declared as abnormal results if data for the developed countries were to be used. The results from this survey support the need to establish reference values using individuals from the population it intends to serve. This will help reduce the inappropriate exclusion of potential clinical trial participants based on reference values derived in the developed countries.

1309

CLINICAL OBSERVATIONS OF HUMAN MONKEYPOX INFECTIONS IN THE DEMOCRATIC REPUBLIC OF THE CONGO

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We describe the results of an observational study of the clinical natural history of human monkeypox (MPX) infections at the remote L'Hôpital Général de Référence de Kole in the rainforest of the Congo River basin of the Democratic Republic of the Congo (DRC). The cardinal observations from 244 subjects enrolled in the study are summarized. All subjects who were positive by pan-orthopox MBG PCR -- utilizing an onsite quantitative real-time assay (LightCycler) -- were also positive by a MPX-specific MGB PCR assay, suggesting that MPX may be the only poxvirus circulating in the area. Sequencing of MPX DNA from one subject's scab showed only 17 nucleoside changes from the MPX Zaire 79 strain (collected in 1979) which was circulating during the WHO clinical characterization studies of 25 years ago when the case-fatality rate (CFR) was about 10%. This is the same isolate USAMRIID has used to develop non-human primate MPX models for drug and vaccine evaluation. The spectrum of disease severity in our study was broad as evidenced by lesion counts ranging from 2 to 8,617, viremia (by PCR) ranging from undetectable to 6.3×10^7 genomes ml/blood, and clinical status ranging from very mild to critically ill. The CFR to date is only 0.9% in subjects aggressively treated with antibiotics, antimalarials, antiparasitics, anti-inflammatory drugs, and IV fluid. A strong correlation appears to exist between maximum lesion count and maximum viral load. Low albumin and total protein levels, as well as elevated liver enzyme and alkaline phosphatase levels, were seen in nearly all cases. In one case, in which viremia was detected before the onset of clinical illness, the maximum viral load occurred before the appearance of lesions and coincident with the onset of symptoms. Fetal demise due to maternal transmission of MPX infection occurred in three of four cases of pregnancy. A high percentage of cases involved transmission within households. The severity of disease within families varied widely without discernable pattern. This observational study is expected to lead to future hypothesis driven studies.

1310

ETIOLOGY OF UNCOMPLICATED FEBRILE ILLNESS AMONG CHILDREN 2 - 59 MONTHS OLD ATTENDING A PRIMARY HEALTH CARE CENTRE IN ZANZIBAR

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A better knowledge of non-malarial fevers is a critical component for improved case management of childhood fevers in the new context of low malaria transmission in Zanzibar. We therefore conducted a health facility based prospective cohort study of the relative frequencies of infectious disease etiologies of uncomplicated febrile illness in North A District, Zanzibar. We enrolled 650 children aged 2 to 59 months old presenting to a primary health care center with confirmed fever (axillary temperature ≥37,5°C) or history of fever within the preceding 24 hours. Patients were clinically managed according to the newly adapted Zanzibar Integrated Management of childhood illness (IMCI) guidelines, which include a Rapid Diagnostic Test (RDT) for malaria. Naso-pharyngeal and rectal swabs were collected for PCR-detection of respiratory tract infection and diarrhoeal etiologies. Further, full blood count, malaria microscopy, C-reactive protein, chest X-rays, urine cultures with antibiotic susceptibility testing, rapid detection of pneumococcal antigen in urine and of Group A Beta-Hemolytic Streptococci from throat swabs were analyzed. Preliminary results from a sub-sample of 292 patients of whom 41% had measured fever show the following distribution of clinical diagnoses (a patient can have >1 diagnosis): 52% pneumonia, 50% upper respiratory tract infection, 22% diarrhea, 8% tonsillitis, 4% skin infection, 3% ear infection and 2% dysentery. After laboratory testing no RDT or microscopy confirmed malaria infection was found. Streptococcus A rapid test was positive in 8% of all patients. Chest x-ray was performed in patients classified as pneumonia according to IMCI (cough and/or difficult breathing and increased respiratory rate). Radiological pneumonia consolidation was verified in 5% of those with pneumonia as initial diagnosis. Urine culture from non-diarrheal patients showed significant growth in 8%. These preliminary results indicate that a majority of the fever episodes were related to respiratory tract infections and diarrhea. However, most IMCI classified pneumonias could not be confirmed with chest x-ray. Further PCR analyses will provide insight into the viral and bacterial etiologies of these febrile episodes.

1311

PRE-TRAVEL VACCINATIONS, PRESCRIPTIONS AND COUNSELING FOR MEDICAL MISSIONARIES AND RESEARCHERS

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Overseas volunteers and researchers face unique risks related to their travel purpose and duration. We sought to characterize these travelers and identify whether they received appropriate vaccinations, prescriptions, and counseling on travel-related issues. Boston Area Travel Medicine Network is a research collaboration of five travel clinics in the greater Boston area that sees ~7,500 travelers per year. We evaluated characteristics of travelers who reported their reason for travel as "missionary/volunteer" or "researcher/student." Between March 2008-July 2010, 15,440 travelers were seen in BATMN clinics. Of these, 1451 (9.4%) were missionaries/volunteers, 1216 (7.9%) researchers/students, and 65 (0.4%) reported both reasons. The median age of all 2,732 was 24 years (range 8-85), and 907 (33.2%) were male. The median travel duration was 21 days (range 1-1,096). Among 4,035 destinations, the most common were Haiti (308; 7.6%), India (228; 5.7%), Kenya (195; 4.8%), China (190; 4.7%), and Tanzania (176; 4.4%). Documentation was available for up to date vaccination status, evidence of immunity, or vaccine receipt at the clinic visit for 1,466 (53.7%) for Td/Tdap, 1,835 (67.2%) for hepatitis B, 2,323 (85.0%) for hepatitis A, 1,088 (39.8%) for influenza, and 199/286 (69.6%) for meningococcus (among persons going to at-risk countries). Commonly prescribed medications included ciprofloxacin (1599; 58.5%), azithromycin (590; 21.6%) and levofloxacin (50; 1.8%) for traveler's diarrhea and atovaquone/proguanil (850; 49.0%), mefloquine (174; 10.0%) and doxycycline (120; 6.9%) for those 1732 persons traveling to chloroquine-resistant malaria risk countries. HIV post-exposure prophylaxis was prescribed for 1,235/1,598 (77.3%) travelers, and 385/1,943 (19.8%) had documentation of tuberculin skin testing. Blood-borne pathogen counseling was documented for 1,235/1,599 (77.2%), evacuation insurance counseling for 1,243/1,878 (66.2%), and rabies or animal bite counseling for 215/1,940 (11.1%). Missionaries, volunteers, researchers and students make up less than 20% of BATMN travelers. Although it is likely that not all of these travelers had direct patient contact overseas, there are still critical gaps in the vaccinations and counseling they receive.

1312

SUCCESSFUL USE OF MODIFIED HEIMLICH VALVE USING PLASTIC GLOVE FOR MANAGING TUBERCULOUS BRONCHOPLEURAL FISTULA

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Sequellae of pulmonary tuberculosis (TB) include pleural effusion, empyema, and bronchopleural fistula. After thoracostomy and appropriate medical therapy, failure of lung reexpansion may signify bronchopleural fistula due to underlying pulmonary destruction, which often results in recurrence of empyema, sepsis and death. We present a 23-year old HIV-negative male in Cameroon with smear-positive TB complicated by empyema and bronchopleural fistula who was successfully managed using a modified Heimlich valve made from the finger of a plastic glove. The patient presented with left-sided empyema, which was drained with a chest tube under water seal. Sputum was positive for acid-fast bacilli, and anti-TB therapy and antibiotics were initiated. After two weeks, a persistent air leak remained and chest radiography showed failure of lung reexpansion. The chest tube was trimmed to 4 centimeters and the 5th finger of a plastic glove with both ends cut was attached to the end and lubricated with Vaseline, which allowed for one-way exit of air and fluid. The tube was left in place for three months to create a chest window, after which the tube was removed, leaving an epithelialized passage between the pleural space and external environment. Scant fluid continued draining from the valve and the chest window during the treatment course but no other complications were noted. Bacteriological cure was confirmed by negative control sputum at two and five months. After 12 months the window had closed spontaneously, and the lung had completely reexpanded. Radiographs illustrate the entire clinical course. The method described here using a widely available resource–a non-sterile plastic glove–to make a modified one-way valve was successfully used for the treatment of tuberculous bronchopleural fistula and persistent pneumothorax. Ongoing drainage of chronic empyema and formation of a chest window is thus possible without advanced thoracic surgical intervention. The glove drain is worth considering in resource-limited settings for this challenging complication of pulmonary TB.

1313

THE BURDEN OF CHRONIC HEPATITIS B IN IMMIGRANTS IN QUEBEC, CANADA: A POPULATION BASED STUDY

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Immigrants have higher mortality from chronic hepatitis B (HBV) and hepatocellular carcinoma as compared to those born in Canada. Despite this disparity there are no screening programs to detect chronic HBV, and HBV vaccine is not routinely given to immigrants after arrival in Canada. This is because there is no population based data describing the burden of chronic hepatitis B in immigrants. To fill this gap we created a cohort of all cases of hepatitis B reported from 1991-2008 in Quebec through linking administrative databases. We linked the MADO (Quebec Reportable Disease database), the MICC (Quebec Landed Immigrant database) and the RAMQ (Quebec provincial health insurance and physician billing database). For incidence rate estimates, denominators for immigrants were obtained from the MICC database (N=757,650 newly arrived immigrants from 1991-2008); for non-immigrants, denominators used 1991, 1996, 2001, and 2006 Quebec census data (immigrants removed). Rates and rate ratios and 95% CI were calculated using the Poisson distribution. 13,889 cases of chronic hepatitis B were reported during the study period. Non-immigrant cases were older (mean age 43.4 vs 33.4 $p < 0.01$) and were more likely male (69% vs 51%, $p < 0.01$). The rate of chronic hepatitis B overall was 10 fold higher in immigrants as compared to non-immigrants [rate ratio; 95% CI = 10.0 (9.7-10.30) and rates/100,000 person years (PY) were 73.9 vs 7.4]. Rates were highest for immigrants from East Asia/Pacific [rate/100,000 PY 95% CI = 280 (268-293)], Sub-Saharan Africa [280 (262-298], Eastern Europe [86 (77-94)]; they ranged from 38-42/100,000 PY for immigrants from South Asia, the Middle East/North Africa and Latin America. Immigrants are at increased risk for chronic hepatitis B and its associated sequelae, including potential transmission to close contacts. Immigrants would therefore likely benefit from screening for chronic hepatitis B and verification of hepatitis B immune status, so that appropriate treatment of chronic infection and vaccination of susceptible contacts can be provided.

1314

CORRELATION OF MALARIA RAPID DIAGNOSTIC TESTING WITH CLINICAL-BASED ALGORITHM IN A RURAL VILLAGE IN UGANDA

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Uganda has the world's highest malaria incidence and mortality. The Engeye Clinic was created in 2006 as a U.S./Ugandan non-governmental organization based in the Ddegeya Village. In this resource poor setting lacking microscopes and trained technicians, rapid diagnostic testing (RDT) was initiated to confirm clinically suspected malaria. Issues facing this community include little to no use of mosquito nets, failure to complete treatment and/or use of paracetamol substituted for malarial treatment by village merchants. The purpose of this study was to evaluate the implementation of a clinical algorithm in a resource-limited setting for the diagnosis of malaria compared to RDT. Over a two week period in February 2010, 344 patients were assessed by the on-site clinician using a clinical algorithm for the diagnosis of malaria. This included fever, chills, sweats, headaches, muscle or abdominal pains, nausea and vomiting for greater than 48 hours with no other obvious cause. RDT was performed on patients meeting clinical criteria for malaria by obtaining whole blood samples using immunographic testing. Treatment for suspected malarial cases was initiated with artemether/lumefantrine based on weight and pregnancy/lactation status. 117 patients met clinical criteria for malaria diagnosis. All clinically diagnosed cases were positive when confirmed with RDT for the detection of parasite specific antigens for *P. falciparum*. The prevalence of malaria as a cause of presenting symptoms was 34% in this cohort. This clinical algorithm was found to be highly specific. The specificity and the positive predictive value of the clinical algorithm was 100% when compared to the RDT in this cohort. In patients without clinical criteria for a diagnosis of malaria there were no positive RDT results. It was determined that the clinical algorithm could be used by rural health care workers to accurately diagnose malaria as misdiagnosis leads to a delay in treatment causing an increased mortality and unnecessary prescription of malarial medications and increased drug resistance.

1315

LYME DISEASE AND FILARIASIS - A *WOLBACHIA* CONNECTION: A CASE REPORT

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Health care providers must consider neglected tropical and regional endemic diseases. Lyme disease, commonly found in North America requires a diagnosis of erythema migrans with confirmatory serology. Lymphatic filariasis, endemic to Africa, is a neglected chronic disease that can be easily overlooked in immigrants coming to North America. We present a case of a 21 year old Liberian male complaining of one week of right knee pain and swelling during the summer. He had a history of malaria and filariasis. He immigrated to Albany, New York eighteen months ago as a refugee from civil war and was taking isoniazid and pyridoxine for a positive tuberculin test. He had no known tick exposure or trauma. He was afebrile and hemodynamically stable complaining of right knee discomfort, warmth, swelling and decreased range of motion. His white blood cell count was 4.7 with 52% neutrophils, 26% lymphocytes, 9% eosinophils and 12% monocytes. C-reactive protein (101 mg/L) erythrocyte sedimentation rate (73 mm/hr) and IgE level (4123 U/ml) were elevated. Knee radiograph showed joint effusion. Synovial fluid aspiration contained 116.5 tho/cmm white cells (95% neutrophils) but gram stain and culture were negative. Lyme PCR from synovial fluid was not submitted for analysis. Lyme C6 peptide was positive as was confirmatory Western blot which demonstrated IgG bands 18, 23, 30, 31, 39, 41, 58 and 93. Antifilarial IgG4 was positive indicative of past or chronic infection with filariasis. A course of Doxycycline was initiated for the management of both acute Lyme arthritis and chronic filariasis. Antifilarial therapy with Doxcycline was directed toward the symbiotic bacteria, *Wolbachia* associated with microfilaria. While it is possible our patient's positive Lyme serology reflected cross reacting antigens to filaria; his clinical presentation was consistent with Lyme associated arthritis. This case is unique in that antimicrobial management of one endemic infection was useful in the management of a geographically separate pathogen.

1316

STRATEGIES TO PREVENT MEASLES, MUMPS AND RUBELLA AMONG NEWLY ARRIVED ADULT IMMIGRANTS AND REFUGEES IN CANADA: A COST-EFFECTIVENESS ANALYSIS

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Adult immigrants are an unrecognized group at risk for measles, mumps and rubella (MMR). They have been over-represented in rubella outbreaks and most cases of congenital rubella (CRS) in Canada have occurred in infants born to foreign-born mothers. Despite this gap, MMR vaccine is not routinely given to adult immigrants after arrival in Canada. We performed a cost-effectiveness analysis to define the optimal strategy to prevent MMR in this population. We constructed a decision analysis (Markov modeling) to compare the cost-effectiveness of four preventive strategies using MMR vaccine, compared to no intervention (status quo). Interventions tested were: 1 and 2) Vaccinating all adult immigrants with either 1 or 2 doses of MMR vaccine without prior serotesting, or 3 and 4) serotesting for MMR then vaccinating those found to be susceptible to one or more of the diseases with either 1 or 2 doses of MMR vaccine. A hypothetical cohort of 250,000 newly arrived immigrants 18 years of age or older was modeled over a 20-year period. The expected number of cases of MMR, associated complications and costs (health care system perspective) for each of the strategies were calculated with 3% discounting for costs and clinical outcomes. Vaccinating all adult immigrants with 2 doses of MMR vaccine without prior serotesting saved 41 million Canadian dollars and avoided 6,362 measles cases, 9 measles deaths, 5,051 mumps cases, 8,181 rubella cases, 1 rubella death and 38 cases of CRS over a 20-year period, as compared to no intervention. Comparing both interventions involving vaccination without serotesting, the 2-dose strategy cost an additional $1.69 per person (total of $422,500) compared to the 1-dose strategy but prevented an additional 273 measles cases, 405 mumps cases, 273 rubella cases and 1 CRS. Both strategies that involved serotesting before vaccination were cost-saving compared to no intervention. However, both serotesting strategies were more costly and less effective than the vaccination-only strategies. All new adult immigrants should be given two doses of MMR vaccine without prior serotesting: this strategy is cost-saving, prevents individual morbidity and mortality, and decreases the potential for outbreaks.

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DETERMINANTS OF ANEMIA AMONG YOUNG CHILDREN IN WESTERN KENYA

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Anemia among children in developing countries is often attributed to iron deficiency caused by low dietary iron intake. However, the causes

of anemia are multi-factorial and interlinked. In sub-Saharan Africa, sickle cell disease (SCD), α-thalassemia, and infections are widespread and are known risk factors for anemia. Data on multiple risk factors for anemia are needed to design more effective prevention and treatment programs. We conducted a cross-sectional cluster survey of 841 children aged 6-35 months in 60 randomly selected villages in Nyando District, western Kenya. Anemia prevalence (hemoglobin 8.3 mg/L) 75%, vitamin A deficiency (retinol binding protein (RBP) 10 mg/L) 30%, reported fever in the last 24 hours 27%, stunting (height-for-age z-score<-2) 30%, wasting (weight-for-height z-score<-2) 3%, sickle cell trait 17%, SCD 2%, heterozygous α-thalassemia genotype 38% and homozygous α-thalassemia genotype 9%. In bivariate analysis, anemia was associated with iron deficiency, vitamin A deficiency, malaria, inflammation, fever, stunting, wasting, homozygous and heterozygous α-thalassemia genotypes, age<30 months, male sex, and low socioeconomic status (SES) ($p<0.05$). In linear regression, accounting for cluster design, the best fit model included TfR, RBP, malaria, CRP, SCD, homozygous α-thalassemia genotype, male sex, age<30 months ($R2= 0.59$, $p<0.0001$). Age <30 months, homozygous α-thalassemia genotype, and CRP modified the relationship between iron deficiency and hemoglobin. Fever, height-for-age z-score, height-for-weight z-score, and low SES were not significantly associated with hemoglobin when included in the best fit model and did not confound the relationship between TfR and hemoglobin. Interventions designed to prevent anemia should utilize an integrated approach, ensuring optimal iron intake while also addressing malaria and other infections.

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COMPLICATIONS OF MONKEYPOX INFECTIONS IN HUMANS

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We report on the complications of monkeypox infections in humans as observed during a 4 year (2007-2011) study at the remote L'Hôpital Général de Référence de Kole in the rainforest of the Congo River basin of the Democratic Republic of the Congo (DRC). The study was conducted jointly by the Institute National de Recherche Biomedicale (INRB) and the US Army Medical Research Institute of Infectious Diseases (USAMRIID). Human monkeypox infections were first identified during the final stages of smallpox eradication when laboratory testing determined that some cases clinically presenting as smallpox were actually caused by monkeypox virus. The present study was conducted at one of the two previous WHO MPX study sites (1981-1986) staffed by the same Spanish medical order of Catholic sisters who have continued the treatment of MPX patients after others in the country stopped hospitalizing such cases. A total of 244 patients were consented and enrolled into our study. Generally, patients presented with fever, chills, sore throat, pox lesions, and general malaise, fatigue. The number of pox lesions ranged from two lesions to more than eight thousands lesions. Complications included death, coma and other neurologic manifestations, co-infections, infected wounds, secondary dermatitis, miscarriages, keratitis, staphyloma, and caseation of eye lesions. The case fatality rate was 0.9% in our study. Case histories with appropriate graphics, including photographs will be presented, when appropriate, to demonstrate findings.

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MID-UPPER-ARM CIRCUMFERENCE IS A USEFUL TOOL FOR ASSESSING NUTRITIONAL STATUS IN YOUNG CHILDREN WITH DIARRHEAL DEHYDRATION

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Undernutrition is implicated in over half of all child deaths related to diarrhea. Cohort studies which assess the relationship between diarrheal disease and nutritional status typically use weight-based anthropomorphic measures. However, such measures are not useful to study the acute nutritional changes induced by diarrhea because children with diarrhea are often dehydrated. Mid-upper-arm circumference (MUAC) is a cheap, simple tool often considered to be a better indicator of acute malnutrition than weight. MUAC measures musculature, a proxy for protein stores. If truly unaffected by interstitial water content, or dehydration, MUAC could be used to assess the acute nutritional changes induced by diarrhea. In February 2011, we initiated a case control study in Mirzapur (Bangladesh), Kolkata (India), and Basse (The Gambia) to determine whether diarrheal dehydration affects MUAC. A case, enrolled at a clinic, was defined as a child 0-59 months old with acute (< 7 days) diarrhea (≥3 abnormally loose stools in the previous 24 hours) who had ≥1 of the following: sunken eyes, skin tenting, dysentery, IV rehydration, or hospitalization, and underwent rehydration therapy. A healthy control matched for age and gender was enrolled at home within 3 days of case enrollment. Weight and MUAC were measured for each case and his/her matched control at enrolment and again 4 hours later. To date, 66 cases and 66 controls have been enrolled. Cases had substantial weight gain within 4 hours, which was significantly more than the controls within 4 hours [mean kg 0.29 (0.16, 0.42) vs. 0.10 (0.02, 0.19) p=0.02]. In contrast, the change in MUAC before and after rehydration in cases was comparable to that in controls measured at two time points 4 hours apart [mean cm 0.03 (-0.01, 0.07) vs 0.01 (-0.01, 0.04) p>0.09]. These preliminary results suggest that rehydration therapy results in weight gain but does not affect MUAC, thus providing a useful tool for epidemiologic studies.

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EMERGING BIOMARKERS FOR THE DIAGNOSIS OF SEVERE NEONATAL INFECTIONS APPLICABLE TO LOW RESOURCE SETTINGS

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Severe neonatal infections are one of the most significant causes of pediatric mortality, resulting in more than 500,000 deaths each year, of which 99% occur in low-resource settings. Compared to clinical algorithms, new point-of-care diagnostics that could distinguish neonates with or without severe infections may have potential to substantially improve the global management of severe neonatal infections. This review sought to characterize promising biomarkers for the diagnosis of severe neonatal infections. Biomarkers extensively reviewed elsewhere (procalcitonin, C-reactive protein, tumor necrosis factor-α, interferon-γ, and interleukin-6 and 8) were not re-reviewed. Hundreds of other biomarkers have been associated with "sepsis"; this review focused exclusively on biomarkers with published performance data for the diagnosis of severe neonatal infections. We identified infant diagnostic

performance data on 21 biomarkers: seven acute phase reactants (Serum Amyloid A(SAA), LPS Binding Protein(LBP), Inter-α Inhibitor Proteins(Iα-Ip), Antithrombin, Soluble E-Selectin, Fibronectin); five pro-inflammatory cytokines (Interleukin-1α, Interleukin-1β, Interleukin-12p70, Interleukin-18, Granulocyte Colony Stimulating Factor(G-CSF)); two anti-inflammatory cytokines (Interleukin-10, Interleukin-1 Receptor Antagonist(IL-1RA)); five chemokines (Growth Related Oncogene α, Interferon-γ-Inducible Protein 10(IP-10), Monokine Induced by Interferon-γ, Regulated upon Activation Normal T cells Expressed and Secreted, Monocyte Chemoattractant 1); one soluble cell surface marker (soluble intercellular adhesion molecule-1); and one molecule involved in triglyceride metabolism (apolipoprotein CII(apoC2)). Seven soluble biomarkers (G-CSF, IL-RA, IP-10, SAA, LBP, IαIp, apoC2), compatible with point-of-care immunodiagnostics (defined as a concentration > 1ng/ml), emerged as promising candidates, with sensitivity and specificity generally > 90% (range 33 to 100%). These biomarkers seem particularly attractive for future prospective studies of diagnostics for severe neonatal infections.

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OUTCOME OF FOUR PREGNANCIES IN CONGOLESE WOMEN WITH MONKEYPOX INFECTION

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The outcomes of four pregnancies in women with clinically apparent, PCR-confirmed, community-acquired monkeypox (MPX) virus infections are described. During 2007 to 2011 we studied the clinical features of human MPX infections in Kole, the Democratic Republic of the Congo. 244 subjects were enrolled of which four were pregnant. The outcomes of these four pregnancies along with the maternal pox lesion counts and the PCR-confirmed viremia were carefully documented. This is the first report of intrauterine demise due to complications of human monkeypox. In Case 1, MPX viremia rose rapidly and abruptly upon cessation of fetal movement at the 18th week of gestation, some 24 days after onset of rash. Marked fetal hepatomegaly and peritoneal effusion (*hydrops fetalis*) were seen at necropsy. In Case 2 a spontaneous miscarriage occurred in a subject without significant viremia or remaining lesions at the 6th week of gestation, 22 days after rash onset. The third spontaneous miscarriage occurred at 7 weeks of gestation and the 10th study day in a mother with over 1,000 lesions and viremia exceeding 10^5 genomes/mL of blood. (No pathology examination was performed and no determination of viral load was made on the aborted material for Cases 2 and 3.) The fourth pregnant subject was enrolled for observation as a healthy family member of an index MPX case. She was then noted to be about 14 weeks pregnant. On her second study day she was noted to have MPX lesions on her genitals. Her lesion count never exceeded 20 and she had a low level viremia by PCR. At her study day 75 follow-up visit (24 weeks gestation), the fetus was alive. Although the sample size is small, we observed a very high abortion rate in cases of maternal MPX infections, but death is not inevitable.

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QUASISPECIES VARIANT ANALYSIS OF A 2010 DENGUE 3 VIRUS FROM KAMPHAENG PHET, THAILAND

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All four serotypes of dengue viruses exist as quasispecies. Quasispecies are described as a spectrum of variants ('candidate genomes'), genetically linked through mutation, creating an interactive population where selection acts on the population rather than the individual variant. We explored an assertion of quasispecies theory that the fitness (ability to infect and cause disease) of a particular viral sequence is determined more by its freedom to mutate than by its ability to replicate. A quasispecies from dengue virus serotype 3 (DENV3) was cloned from a single mosquito collected within a cluster of human dengue infections (100 meter radius) in Kamphaeng Phet, Thailand, in 2010, to understand diversity and mutational effects apparent in the population. Sequences were combined with other published DENV3 sequences and maximum likelihood phylogenetic analysis revealed quasispecies populations removed from the baseline 'consensus sequence' diversity of human DENV3 circulating in Thailand. Mutational analysis showed a high proportion of nonsynonymous mutations and 2.8% of the population was evolving faster per site than average despite overall low diversity. Forty-four percent of the sequences were under positive selection while 19% were under purifying selection. Quasispecies analysis identified amino acid substitutions that have been reported to lead to phenotypic changes in viral like particle assembly, prM/E protein production, glycosylation and/or antibody binding ability. Other uncharacterized amino acid substitutions identified are predicted to be deleterious. The diversity of the quasispecies suggests there are variants with altered abilities to infect and disperse with overall diversity being constrained in the mosquito. An altered ability to infect or disperse will potentially affect how the population responds to selective pressures such as innate immunity and vaccine implementation.

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VALIDATION OF DENGUE SEVERITY PREDICTIVE ALGORITHMS DERIVED FROM PRIMARY CARE AND HOSPITALIZED CASES IN AN ADULT SECONDARY CARE COHORT

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Dengue is the most prevalent arthropod-borne infection worldwide. In well-resourced centers where diagnosis can be rapidly established, the next crucial step is to triage for appropriate care. Singapore has primarily adult dengue disease and recent epidemics have led to development of predictors to guide admission to secondary care. We validate three algorithms in a prospective cohort of 137 laboratory confirmed adult cases referred to a hospital-based dengue clinic. Cases that have already fulfilled severity criteria at presentation are excluded from analysis. First, the decision tree classifier developed from a febrile (<=72hrs) primary care cohort with laboratory-confirmed dengue fever, as reported previously: a cycle threshold of real time reverse-transcriptase polymerase chain reaction <=20.9 with positive dengue IgG at presentation, or platelet

count of <108 000/mm3. Reported sensitivity (Sn) was 78.2% and specificity (Sp) 80.2% in predicting a platelet nadir of 50 000/mm3. In our cohort, Sn/Sp=88.9%/66.7% in an identically defined subgroup (n=30), with no significant difference between previously published and our Sn/Sp. Second, comparison was made with a decision tree developed from a retrospective hospitalized cohort to predict dengue hemorrhagic fever (DHF) (Lee et al Trop Med Int Health. 2009 Sep;14(9):1154-9). Reported Sn/Sp=100%/46% using any of a history of bleeding, serum urea >4 mmol/L, or serum protein <=67g/L. In our cohort (n=115), Sn was significantly lower at 85.7% but difference in Sp at 49.4% was not significant. Last, the predictive equation for DHF using history of bleeding, serum urea, serum protein and lymphocyte proportion from the same cohort (Lee et al, J Clin Virol. 2008 May;42(1):34-9) had Sn/Sp=97.6%/60.3%. Our Sp was significantly lower at 32.2% but Sn was not significantly different at 100%. While our cohort was more severe than the hospitalized training cohort and the primary care cohort (24% vs 4% vs 2.6% DHF), it is reassuring that sensitivities remain high. Given the wide spectrum of dengue disease and varying presentations in different populations, a thorough exploration of the utility of prognostic algorithms taking into account population and clinical factors such as time to presentation will be required to safely triage dengue patients. We showed that the utility of predictors may vary even within the same country depending on source of patients.

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RAPID DIAGNOSIS OF DENGUE IN A HOSPITAL-BASED COHORT

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Accurate and rapid dengue diagnosis is vital to triage and management. The World Health Organisation (WHO) proposed in 2009 an updated clinical definition of probable dengue replacing 1997 criteria for suspected dengue fever. Definitive laboratory diagnosis of dengue is not always possible, and newer methods such as testing NS1 antigen are undergoing evaluation. We prospectively enrolled 205 adult suspected dengue cases referred to the Communicable Disease Centre, Singapore to comprehensively evaluate methods for rapid diagnosis of dengue. Clinical and laboratory criteria were evaluated, including daily PCR, NS1 antigen, and IgM/IgG serology for those positive by PCR or NS1 on presentation. Confirmed dengue cases (n=142) were positive by PCR/NS1 or by IgM seroconversion by ELISA at 3-4 weeks. Non-dengue cases (n=20) were negative by PCR, NS1 and IgM ELISA in paired sera. Forty-three cases could not be assigned an acute dengue diagnosis because of a lack of paired sera or elevated IgM/IgG without seroconversion. The sensitivity (Sn) of PCR at presentation (median fever duration 5 days, range 2-9 days) was 70.4% and specificity (Sp) 100%. Median duration of viremia was 6 days (range 3-11 days). For NS1, Sn/Sp=89.4/100%, with median duration of antigenemia of 7 days (range 2-10 days), significantly longer ($p<0.001$) than median viremic duration. Using <=5 days of fever as a cutoff for early illness, the Sn/SP of PCR (n=84) was 88.9/100% vs 51.4/100% late in illness (n=78), compared to NS1 of 90.3/100% early and 88.6/100% late in illness. Only 2 cases (1.4%) were detected only by IgM seroconversion with negative results by PCR and NS1. WHO 1997 criteria for dengue fever had Sn/Sp=98.6/20.0% while the recent 2009 criteria for probable dengue Sn/Sp=97.9/20.0%. Laboratory diagnosis using NS1 antigen had consistently high Sn/Sp, with markedly improved Sn compared with PCR after day 5 of fever ($p<0.001$), and was positive for a mean of 1.1 days longer than PCR. Assessing seroconversion did not substantially increase the sensitivity of diagnosis in hyperendemic Singapore. Both clinical guidelines had similar test characteristics: very sensitive but with poor specificity in a cohort of referrals for suspected dengue.

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GENETIC DIVERSITY OF DENGUE VIRUS IMPACTS TO THE DETECTION SENSITIVITY OF RT-PCR BASED METHOD, ONE CAUTION FOR METHOD DEVELOPMENT AND QUALITY CONTROL ASSESSMENT

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Dengue virus (DENV), transmitted by Aedes mosquitoes, causes the disease in 50-100 million people per year in tropical and subtropical regions worldwide. Four DENV serotypes (DENV-1 to -4) can cause infections ranging from asymptomatic or mild febrile illness to severe hemorrhagic disease. Various RT-PCR techniques have been developed for rapidly detecting and typing DENV. The rapid diagnosis allows early initiation of patient care and specific preventive health measures. RT-PCR is also a useful diagnosis tool for surveillance studies. Our laboratory has used the modified Lanciotti's conventional RT-PCR method as one of the diagnostic tests for DENV since 1994. The method was evaluated and classified as an optimal method for DENV diagnosis and surveillance by an international External Quality Control Assessment (EQA). However, the analysis of PCR results of 13,532 dengue confirmed cases by ELISA tested over 11 years (2000-2010) showed 17-42% negative PCR results. Among these negative PCR results, 36% were from acute sera from patients within 0-4 days of illness onset, typically a viremic period with a high percentage of virus isolation. We tested 300 samples from patients with dengue confirmed by ELISA and with negative conventional PCR results with in-house TaqMan RT-PCR which was classified as a 'need of improvement' method by EQA as part of a quality improvement effort. One hundred and eighty samples (60%) showed TaqMan positive results including 129 DENV-1 (43%), 39 DENV-2 (13%), 4 DENV-3 (1.3%) and 8 dual infections of DENV-1 and -2 (2.7%). Sequences of conventional PCR primers binding sites of 9 TaqMan positive samples including 4 DENV-1, 3 DENV-2, and 2 DENV-3 revealed points of mismatch between primers and templates that likely effected the sensitivity of the detection. These data indicated that the genetic diversity of DENV impacts the sensitivity of RT-PCR based methods, a critical concern during method development and quality control assessment.

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CONSIDERATIONS FOR CHANGING PRNT DENGUE 4 REFERENCE VIRUSES: SUB-OPTIMAL IMMUNITY TO DOCUMENTED INFECTIONS

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The plaque reduction neutralization test (PRNT) is considered to be the "gold standard" to characterize and quantify circulating levels of anti-dengue virus (DENV) neutralizing antibody. The PRNT is used to define the immunogenicity of dengue vaccine candidates, support dengue seroepidemiologic and pathogenesis studies. Despite numerous efforts to standardize the assay and normalize data to better compare data between studies and natural and vaccine infections, there are several sets of reference viruses around the world. The Armed Forces Institute of Medical Sciences in Bangkok utilizes DENV-1 (16007), DENV-2 (16681), DENV-3 (16562) and DENV-4 (1036) reference strains. In 2006 the dominate

serotype in circulation in Thailand was DENV-4. In cohort studies we observed poor or absent PRNT titers using the 1036 DENV 4, genotype 3 strain (originally isolated in 1976 in Indonesia) to documented DENV-4 infections. New candidate DENV-4 reference viruses were selected from isolates collected in the last 10 years. These viruses were tested using a bank of sera from documented DENV-4 infections including homologous sera from the individuals from which the strains were isolated. A candidate reference strain was selected based on PRNT titers achieved, low cross reactivity, and the ability of the virus to produce large well-formed plaques. More than 300 samples were tested with the old and new reference virus. Geometric mean titers were increased 4.2 fold. Using the new reference virus enabled identification of additional inapparent infections in cohort studies and has enhanced our ability to characterize the DENV-4 immune response. This study illustrates the need to continuously monitor the performance of viral strains in reference assays. Furthermore, this data suggests that dengue viral evolution may have a profound effect on tests that utilize reference strains.

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SAFETY OF A RECOMBINANT LIVE ATTENUATED TETRAVALENT DENGUE VACCINE IN HEALTHY ADULT VOLUNTEERS

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Dengue (DEN) virus threatens over half the world's population, causing debilitating dengue fever, dengue hemorrhagic fever and dengue shock syndrome leading to over 20,000 deaths every year. DENVax is a tetravalent live attenuated dengue vaccine that is based on the DEN-2 PDK-53 genetic backbone. DEN-2 PDK-53 has been tested previously in humans and was found to be safe and immunogenic. Recombinant DENVax-1, DENVax-3 and DENVax-4 strains were generated in which the prM and E genes of PDK-53 were substituted with those of DEN- 1, -3 or -4 viruses. These recombinant viruses retain the genetic attenuation markers present in PDK-53 and direct the immune response to the other three serotypes. A single center, placebo-controlled, randomized study assessing the safety, tolerability of tetravalent DENVax formulations was performed in Rionegro, Colombia, a high altitude area with no *Aedes aegypti* and no dengue exposure. One of two dose levels (low or high) of DENVax was administered subcutaneously or intradermally to healthy male and female subjects with no pre-immunity to flaviviruses. Two doses of DENVax or placebo were administered, separated by an interval of 90 days. Safety was assessed as the frequency and severity of adverse events through physical examination, injection site examination, lab examinations, and subject diary cards. Clinical laboratory assessments included serum chemistry, hematology and urinalysis. The safety data demonstrate that both tetravalent formulations were well-tolerated by either route of administration. To date, the most frequent adverse events were local reactogenicity at the injection site for both dose levels and both routes of administration. Systemic adverse events were mild to moderate headache, muscle pain, nausea and fatigue. There were no meaningful laboratory changes. This study highlights the safety of the tetravalent DENVax formulations in healthy adults. Further clinical trials to assess safety, tolerability, and immunogenicity in other age groups and in dengue exposed individuals are being planned.

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DEVELOPMENT OF A RECOMBINANT TETRAVALENT DENGUE VACCINES (TDV) THAT LINKS INNATE AND ADAPTIVE IMMUNITY

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We have previously demonstrated that the domain III of West Nile virus envelope antigen (EIII) fused to flagellin of *Salmonella typhimurium* (STF2, a TLR5 ligand) is immunogenic and efficacious against lethal WNV infections in mice (McDonald *et al*., 2007, J. Infect Dis. 195, 1607-1617). To develop a tetravalent dengue vaccine, we have designed, purified, and evaluated similar and alternative flagellin-EIII fusion vaccine formats, which differ in the site of antigen attachment to the flagellin. These fusion proteins can be efficiently and economically manufactured in *E. coli* fermentation systems. Here we report immunogenicity results of recombinant dengue vaccine candidates in monovalent, bivalent, and tetravalent formulations. BALB/c mice were immunized s.c. three times at 2 or 3 week intervals, and bled at various times post boost. In an efficacy study, AG129 mice lacking receptors of types I and II interferons were immunized with two or three doses of a monovalent DENV-2 vaccine candidate, and challenged with 2,100 LD_{50} of DENV-2 (strain NGC). Serum neutralizing antibody titers were determined by 50% plaque reduction neutralization test ($PRNT_{50}$). Survival rates, weight changes, and viremia, as measured by qRT-PCR, of infected mice were determined. Our results indicated that immunizations of BALB/c mice with these vaccine candidates at doses of 2-15 µg elicit robust homotypic neutralizing antibody responses. Furthermore, a monovalent DENV-2 candidate conferred partial protection against a lethal DENV-2 challenge and significantly reduced viremia and weight loss in infected AG129 mice. The DENV-2 candidate was also found to elicit high $PRNT_{50}$ titers in rabbits. Finally, BALB/c mice immunized with tetravalent dengue flagllin-EIII formulations developed strong neutralizing antibodies to all 4 serotypes of DENV (GMTs of $PRNT_{50}$ = 200 - 3000). In conclusion, VaxInnate flagellin-EIII vaccine candidates are highly immunogenic in mice and rabbits and are effective in protecting AG129 mice against a lethal DENV-2 challenge, thereby justifying further development of a TDV.

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IDENTIFICATION OF HOST FACTORS THAT INFLUENCE DENGUE VIRUS INFECTION IN HUMAN PRIMARY MONOCYTES AND MONOCYTE-DERIVED DENDRITIC CELLS

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Dengue virus (DENV) is a flavivirus in the family flaviviridae that infects up to 50-100 million people per year, with 2.5 billion people at risk. The burden of disease is significant, with a clinical primary infection manifesting as fever, rash, severe headaches, and intense myalgia and arthralgia that persist for approximately one week. Elucidating the interactions between host cell proteins and the dengue virus is critical to the development of targeted and effective antiviral drugs. Learning the details of these interactions will be essential to be able to rationally design drugs targeting viral proteins, or to identify compounds that will interfere with host processes critical to DENV infection. The first step towards this end is to identify which host proteins interact with the dengue virus in a clinically relevant system. Proteomic evaluations of host cells following

dengue virus infection have been performed in liver cells and endothelial cells but not the described primary target of dengue virus infection, primary human monocytes and dendritic cells. We used a proteomics-based approach to identify host factors relevant to dengue virus infection in primary human monocytes and monocyte-derived dendritic cells. After infecting these cells with DENV serotype 2 strain 16681 we compared their proteome to that of uninfected cells using the Beckman Coulter PF2D system, a fluid-based system analogous to a 2D-gel that separates proteins by isoelectric point (pI) followed by hydrophobicity. After comparing infected cells to uninfected cells we found approximately 75 proteins that either increased or decreased in abundance by greater than 2.5 fold in the presence of DENV. These unidentified proteins were then subjected to Mass Spectrometry analysis. Proteins down-regulated in the presence of DENV in both monocytes and monocyte-derived dendritic cells were chosen for further analysis to elucidate their role in the pathogenesis of dengue virus.

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SEROTYPE-SPECIFIC DENGUE VIRUS CIRCULATION AND DENGUE DISEASE IN BANGKOK, THAILAND FROM 1973 TO 2010

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Since 1962, the Queen Sirikit National Institute of Child Health (QSNICH) and AFRIMS have cooperated in a mutual public health effort to accurately diagnose dengue infections including serologic determinations of antibody patterns and identification of dengue serotypes. The epidemiologic data included all patients admitted to the dengue ward of QSNICH with suspected dengue fever and dengue hemorrhagic fever who were subsequently proven to have dengue infection by serology or virus detection. Available data from 1973 t0 1999 have been analyzed and published previously (Nisalak et al, 2003). We report on data for the expanded years from 1973 to 2010 including many more cases of DENV-4 infection than were observed previously. Findings that were reconfirmed from the previous report: 1) primary cases are increasing relative to secondary cases; 2) symptomatic primary cases were most likely due to DENV-1; 3) Primary non-infant hospitalized cases were less severe than secondary non-infant hospitalized cases. The mean age of DHF cases are noted to be increasing. In 1973-1982, the mean age of primary and secondary infection was 4.5 years and 8.0 years, respectively. In 2001-2010, it was 6.1 years and 8.0 years. These findings highlight the longitudinal epidemiology of dengue over a uniquely extended period of observation. Further spatial analysis is planned to elucidate transmission dynamics.

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THE ROLE OF ROS SIGNALING IN MOSQUITO CELLS THAT SURVIVE DENGUE 2 VIRUS INFECTION

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Dengue virus (DENV) is naturally transmitted by *Aedes* mosquitoes between humans and replicates efficiently in mosquito as well as in mammalian cells. However, the fate is distinct between the two types of cells in response to the infection. Cytopathic effects (CPE) in mosquito cells are generally trivial compared to that occur in mammalian cells that usually end up with apoptosis. In spite, production of ROS resulted from mitochondria dysfunction occurs in both cell types. It was demonstrated that the survival of mosquito cells is beneficial form up-regulation of genes related to antioxidant defense, such as glutathione S transferase (GST). The anti-apoptotic effect plays a role as the second defense system on protection of mosquito cells from DENV infection. It was eventually regulated by inhibitors of apoptosis (IAPs) that are the upstream regulators of caspase 9 and caspase 3. C6/36 cells with double knockdown of GST and IAP showed a synergistic effect on activation of caspases, causing a higher rate of apoptosis rate (>20%) than those with knockdown of each single gene (~10%), after infection by DENV. Compared with mammalian cells, residual H_2O_2 after anti-oxidation in DENV-infected C6/36 cells may serve as the signal up-regulating the expression of IAP. Taken together, two defense systems including antioxidant defense and anti-apoptotic effects exist in mosquito cells; which were linked by ROS, *i.e.*, H_2O_2 signaling.

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EPIDEMIOLOGY OF DENGUE IN MALAYSIA

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The first major epidemic of dengue fever in Malaysia occurred in 1973, and since that time dengue epidemics have become more frequent, and more virulent. We analyzed data collected by the Ministry of Health Malaysia between 2001-2010 and describe increasing incidence from 68.2/100,000 in 2001 to 159.7/100,000 in 2010, with a spike of 176.5/100,000 in 2008. Analysis of surveillance and notification data collected between 2005-2010 showed that the DHF/DSS:DF ratio was 1:19 in 2005 and 1:10 in 2010, with higher rates of severe disease in secondary versus primary infections. The mean age of DHF/DSS cases was 28 years. Age-specific incidence was highest in adults aged 20-29 years and incidence rates were higher in males, with a male to female rate ratio of 1.397 (95% CI: 1.390 - 1.404; p<0.005). Between 2005-2010 there was a shift to increased transmission in urban settings, with an increase in urban:rural rate ratios from 1.5 in 2005 to 2.0 in 2009, based on urban incidence rates of 170.4/100,000 compared to 98.7/100,000 in rural areas with an urban and rural rate ratio of 1.727 (1.719 - 1.735; p<0.005). Analysis of approximately 700 virus isolates collected between 2005-2010 showed that all 4 DENV serotypes circulated in Malaysia during this period, with all four serotypes detected in each year. DENV-1 genotype I, a virus which has circulated in Southeast Asia since 2003, reemerged as the predominant serotype in 2010. We describe a marked increase in dengue epidemic activity in Malaysia within the last decade, characterized by hyperendemic transmission of all four dengue serotypes, increasing rates of severe disease and increasingly urban transmission.

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AN ISLAND-WIDE DENGUE EPIDEMIC - PUERTO RICO, 2010

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Dengue, a potentially fatal febrile illness caused by four mosquito-transmitted dengue viruses (DENV-1-4), is endemic in Puerto Rico. In January, 2010, the number of suspected dengue cases reported to the Puerto Rico Department of Health/CDC passive dengue surveillance system exceeded the epidemic threshold. To characterize this epidemic, surveillance data were used to describe all reported cases. Suspected cases were patients with a serum specimen submitted for dengue testing. Laboratory-positive cases had (i) DENV identified via reverse transcriptase polymerase chain reaction (RT-PCR) in an acute specimen, and/or (ii) anti-DENV IgM detected in a convalescent specimen. Laboratory-negative cases had no anti-DENV IgM in a convalescent specimen and an acute specimen that was either RT-PCR-negative or not submitted. Indeterminate cases were RT-PCR-negative in an acute specimen and had

no convalescent specimen submitted. In 2010, 23,622 suspected dengue cases were reported, of which 10,956 (46.4%) were laboratory-positive, 2,588 (11.0%) were laboratory-negative, and 9,999 (42.3%) were indeterminate. Of 7,424 RT-PCR-positive specimens, DENV-1 (69.0%) and DENV-4 (23.6%) were detected more frequently than DENV-2 (7.3%) and DENV-3 (<0.1%). Of all laboratory-positive cases, nearly half (46%) were adults ≥ 20 years of age, 4,173 were hospitalized, and 254 had met the 1998 WHO definition of dengue hemorrhagic fever. Enhanced surveillance detected 38 laboratory-positive dengue deaths, yielding an incidence of 3.5 laboratory positive deaths per 1,000 laboratory positive cases; 89% of these deaths were in adults. The 2010 epidemic was long in duration, high in magnitude, and resulted in the most dengue-related deaths since surveillance for dengue began in Puerto Rico in the late 1960's. For this reason and using lessons learned from the 2007 epidemic in Puerto Rico, CDC implemented an initiative to train Puerto Rico clinicians in the management of dengue patients to minimize morbidity and mortality in future epidemics.

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SEROLOGICAL SURVEY OF DENGUE INFECTIONS AMONG INDIVIDUALS IN RAYONG, THAILAND

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Dengue and DHF have been a major public health problem in Thailand over the past 50 years. Clinical dengue (DF and DHF) has traditionally affected children with rare cases among adults. Even though the incident number of DHF cases does not seem to have decreased, a shift towards older age groups has been observed over the past years. The reasons for this shift have not been elucidated. We report the results of an age-stratified serological study conducted among school aged children living in the district of Mueang Rayong in Rayong, Thailand. Schools and classrooms were sampled probabilistically from all schools serving the district. A total of 1812 children (approximately 140 per age group) from 25 schools were enrolled and provided a blood sample. Samples were analyzed using single dilution neutralization testing (SDNT), an assay that differentiates between primary and secondary infection and is serotype specific for those subjects that have only been exposed to one dengue serotype. Preliminary results (n=720) show that 72% (95%CI 61-82%, n=71) of children have been exposed to dengue by age 10 years and that 16% (95%CI 2-30%, n=25) of children have only undergone primary exposure by 18 years of age. These results are significantly different from a similar study conducted by Sangkawibha et al. in Rayong in 1980, where 97% (95%CI 93-100%, n=65) of sampled children were seropositive by age 10 years. This change is consistent with an overall decrease in transmission intensity (force of infection) of dengue in Rayong over the last years. Analysis of the full dataset will explore geographic heterogeneity and factors associated with seropositivity.

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ENHANCED SURVEILLANCE FOR FATAL DENGUE IN PUERTO RICO

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Dengue has been endemic in Puerto Rico (PR) for four decades, and data suggests disease severity increased. Trends are monitored by the passive dengue surveillance system (PDSS) operated by the Centers for Disease Control and Prevention's (CDC) Dengue Branch and the PR Department of Health (PRDH). Suspected cases, including fatals, are reported to the PRDH or the PDSS, and reporting to PDSS requires submission of a serum sample. Prior to 2010, fatal cases were also detected by review of death certificates (DC) that had dengue as cause or contributing cause of death. However, a 2007 evaluation of PDSS found limited ability to detect deaths because it collects data early in the course of disease and few clinicians revise the PDSS report when a patient dies (<10%). In addition, <50% of laboratory-confirmed deaths identified by PDSS had "dengue" on their DC. Deaths are difficult to diagnose as many die on day 4 or 5 of illness when standard diagnostic tests are often unable to detect dengue virus (DENV) or IgM anti-DENV. Although tissue diagnosis is more sensitive in fatal cases, <40% of cases in 2007 had tissue submitted and collection was not systematic. To improve detection and diagnosis of fatal dengue cases, CDC developed an enhanced surveillance system in collaboration with Institute of Forensic Sciences of PR and CDC Infectious Diseases Pathology Branch. All patients who die with a dengue-like, acute febrile illness are identified through weekly calls to hospitals and at death investigation, autopsy, or DC review, and tissue specimens and autopsy findings are collected. During 2010 epidemic, 122 suspected fatal cases were identified of which 38 were laboratory-confirmed, more than twice the number previously identified. The majority (65%) of suspected cases submitted tissue, a higher percent than in any other year which notably reduced the proportion of laboratory-indeterminate cases. Only 16% of the 38 laboratory-confirmed cases had dengue on their DC. In spite of conventional wisdom, dengue deaths appear to be under-reported even in endemic areas.

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DENGUE FEVER DURING THE 2005 AND 2007 EPIDEMICS IN PUERTO RICO: EXPERIENCE OF A TERTIARY LEVEL HOSPITAL

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Dengue Fever is now the most important arthropod borne disease worldwide. It is caused by four serotypes of the Dengue virus. It can be a non-specific febrile illness without complications or it can progress to severe disease with plasma leaking, shock, bleeding and severe organ damage. In Puerto Rico there have been island wide epidemics since 1915 and these have increased in frequency and severity over the past 20 years. Until 2010, the 2007 epidemic had been one of the largest and most severe, presenting many diagnostic and management challenges. The goal of our study was to characterize the epidemiologic and clinical features of pediatric Dengue Fever admissions to the Hospital Episcopal San Lucas in Ponce during the 2007 epidemic and compare with the 2005 outbreak. The study was based at Hospital Episcopal San Lucas, a tertiary level hospital in southern Puerto Rico from January to December of 2007. Pediatric residents reviewed the records of 163 cases with a diagnosis

of Viral Illness with Thrombocytopenia that met the World Health Organization definition of Dengue Fever. The study included a comparison with Dengue Fever admissions (n= 71) during the 2005 outbreak, when data was available. Female/male distribution was 49%/51% in 2007 and 44%/56% in 2005. About 90% of patients were between the ages of 3 to 18 years in 2007 and in 2005. Platelet counts under 50,000 were more frequent in 2005 (74.3% versus 38.7%). During the 2007 epidemic 100% patients presented with a history of fever, 59% had vomiting and 39% had abdominal pain. Maintenance hydration therapy in 2007 included fluids that are considered hypotonic in 65% and isotonic in 16% of cases. Our study reveals that the pediatric age group most frequently affected in both epidemics was the school age and adolescent. Difficulties in the diagnosis during the 2007 epidemic may be related to less thrombocytopenia and the presence of gastrointestinal symptoms. Plasma leaking complications in 2007 may be due to disease severity complicated by hypotonic hydration fluids.

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EVALUATION OF COMMERCIALLY AVAILABLE DENGUE DIAGNOSTIC TESTS: NS1 AND IGM RAPID TESTS AND NS1 ELISAS

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The World Health Organization (WHO) and the Pediatric Dengue Vaccine Initiative (PDVI) established a network of 7 worldwide laboratories with dengue diagnostic expertise to provide an independent evaluation of currently available commercial kits for dengue diagnostics. Each laboratory contributed serum samples to develop a well characterized panel for testing dengue non-structural protein 1 (NS-1) antigen and dengue virus IgM antibodies (IgM anti-DENV). Asia and America region panels of similar composition were developed to evaluate NS1 and IgM anti-DENV test kits. The NS1 combined panel consisted of 192 sera from 147 patients defined as dengue positive and 142 negatives by culture or PCR. The IgM anti-DENV panel had 228 positive and 155 negative sera as defined by reference MAC-ELISAs at Mahidol University and CDC. Seven companies submitted 3 NS1 microplate ELISAs, 1 IgM microplate ELISA, 4 NS1 rapid diagnostic tests (RDT) and 4 IgM anti-DENV RDTs for evaluation. All kits were evaluated at the network sites using region-specific panels. The panel was coded so that technicians performing the evaluation were blinded to the reference assay results. Evaluation results were analyzed to determine sensitivity, specificity, inter-laboratory agreement, inter-reader agreement, lot-to-lot variation and ease-of-use. Preliminary results showed that the 3 NS1 ELISAs had sensitivities ranging 52-46% and specificities of 71-80 %. In comparison, the NS1 RDT had sensitivities ranging 28-59% and specificities of 71-76%. Of the 4 IgM RDTs, sensitivities ranged 52-95% with specificity from 83-90%. Sensitivity of IgM ELISA was 96% and specificity 84%. The range of acceptable sensitivity or specificity from the combined Asia/America panels is being considered by a panel of experts convened by WHO/PDVI. The threshold for acceptable performance may vary by the purpose of testing and by local epidemiology and final recommendations will be distributed as a report upon completion of the analysis.

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A RETROSPECTIVE GEOCODING STUDY ON THE IMPACT OF URBANIZATION ON INCIDENCE RATES OF DENGUE FEVER WITHIN BORNEO, KUCHING DIVISION, FROM 2009 TO 2010, USING ARCGIS

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Review of available data indicates that the impact of urbanization has not been extensively analyzed as it effects disease transmission of Dengue. Understanding urbanization, its meaning on the vector habitat, and how it contributes to disease transmission is vital. The districts of Bau, Lundu and Kuching, all have documented cases of Dengue and the repository of epidemiological data at the Divisional Health Office in Kuching, Sarawak is extensive. By incorporating Geographic Information Systems (GIS) to retrospectively analyze urbanization within these districts of Sarawak, and their cases of DF and DHF from January 2009 to June 2010, projections of the impact of urbanization on the vector distribution and density can be extrapolated and aid in the development of future prevention strategies. Spatial and descriptive analyses methods were used as was two sample t-test. Specific to Kuching District there is a direct correlation between urbanization and an increase in the vector responsible for dengue. Additionally, urban areas experienced higher rates of disease transmission. Three areas of the Kuching, Sarawak Division will be selected; Kuching, Bau and Lundu Districts. The selection criterion is based on the presence of Aedes mosquito breeding, and the frequent reporting of cases within these areas. All clinically diagnosed dengue cases, reported to the Health Office from Jan 2009 - Jan 2010, from Kuching Division will be included for GIS geocoding. Only geocodable addresses using ArcGIS will be included. An interactive method using data abstraction from case records, use of additional maps and street reference will be used to determine geocode within ArcGIS. For Kuching Division, an overall description of area (urban, semi-urban or rural), basic facilities, and geographical profiles, population, population density, and average annual population growth rate as provided by the Malaysian Census will be included. Incorporation into ArcGIS will be based on census tract files availability for 2009 - 2010. Clearly, supplemental GIS studies are warranted and should include habitat, climate and soil moisture modeling and variability in entomological parameters. In order to mitigate further disease transmission, an integrated approach is required for the endemic areas and developing areas of SE Asia.

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CHARACTERIZATION OF DENGUE FEVER IN SCHOOL CHILDREN IN MEDELLIN, COLOMBIA

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Dengue fever is the arboviral disease with the most significant impact in public health. In Colombia, the largest outbreak ever recorded occurred in 2010 with at least 151,774 cases. Understanding the factors and rates of transmission in schoolchildren are needed towards characterizing the burden of disease in the community and defining strategies for epidemic control. In this study, the incidence, seroprevalence and circulating serotypes of DENV were determined in schools from three different neighborhoods of Medellin (San Javier, Poblado and Laureles). A cohort containing 2,340 volunteer students from two public and one private school including primary and high schools (ages 5-19) was established. In the cross sectional study, blood samples were obtained from all admitted students and specific dengue IgM ELISA were performed. The longitudinal study involved surveillance of absenteeism of enrolled students due to febrile illness shorter than 7 days. Standardized physical exam were performed and venous blood samples were obtained from ill

students during both acute and convalescent stages. Dengue diagnosis was confirmed using RT-PCR and IgM ELISA. Among the 2340 students enrolled, 53% were women and students of all grades were represented. In the cross sectional study, 69 (2.9%) students were positive for IgM antibodies. Their mean age was 11.4 years (range = 5 to 19 years) and the distribution of cases by sex was the same. In the longitudinal study, among the 146 students declared ill because of absenteeism due to febrile illness, 12 (8.2%) had IgM antibodies against dengue and DENV-1 serotype was detected by RT-PCR in three of them. The highest frequency of DENV seropositive cases was detected in San Javier's school (3.9%), followed by the Laureles' school (3.4%). In this first phase of the study, a high incidence of dengue fever was found in school children mirroring the large outbreak experienced in 2010.

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ASSOCIATION OF POLYMORPHIC VARIANTS IN TNF-A, IL-6 AND IFN-Γ GENES IN PATIENTS AFRO-DESCENDANT AND MESTIZOS WITH DENGUE INFECTION, COLOMBIA

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Dengue is an important problem of health public in tropical and sub-tropical countries. On the other hand, the response to dengue infection is influenced by the genetic background of the host. In this study was evaluated the association of polymorphic in TNF-α, IL-6 and IFN-γ genes between two ethnic groups with dengue. The study was carried in Antioquia and Chocó, two departments of Colombia. The study population consisted of 122 Afro-descendents patients and 104 mestizos patients with dengue infection. The ethnic group was based using 19 ancestry informative markers (AIMs). The clinical form more frequently was dengue fever (90.3%). Comparisons between ethnic groups showed significant differences. In Mestizos was significantly more frequently the cases of dengue hemorrhagic fever (16.3% vs. 4.1%, p=0.003), more patients had thrombocytopenia (66.7% vs. 47.3%, p=0.012) and more patients were hospitalized (63.5% vs. 23.8%, p< 0.000) compared with afro-descendents patients. The difference between the average of ancestral component was obtained with an ANOVA, showing that the European component had effect above IL6 genotypes distribution, G/A (0.213 ± 0.135) G/G (0.3 ± 0.216) p=0.038. The African component was higher in dengue fever (0.526 ± 0.26) than in dengue hemorrhagic fever (0.376 ± 0.234), it confers protection until 50% (OR=0.49; IC=0.27-0.9; p=0.023). Of the 5 candidate loci (IL64589, TNF -376, CD209-336, INF-Y4100, INF-Y 78), in INF_Y G4100T and TNFα G376A, minor allele frequency were lower to 5%; at this respect, in an association analysis, was compared allelic and genotypic frequencies, finding significant differences for IL6 4589 locus between predefined disease groups, been homozygous G/G frequency higher in DF than in DHF (83% vs. 68%, respectively). This was the first evidence that IL6 polymorphism can be implied in protection/susceptibility to the infection for dengue virus. These results provide evidence about the susceptibility genetic to the infection for dengue virus. However further studies are still necessary.

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ASSOCIATION BETWEEN PRE-EXISTING DENV ANTIBODY AND THE OCCURRENCE OF SYMPTOMATIC ILLNESS DUE TO DENV-4 INFECTION, IQUITOS, PERU

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Dengue fever is caused by infection with any of four distinct viral serotypes (DENV-1 through DENV-4). Antibody induced by infection with one serotype can influence the clinical outcome of subsequent infections. In general, however, details of modifying effects are poorly understood, even though they are potentially critical determinants of transmission dynamics and disease severity. We analyzed data from an on-going longitudinal study of DENV transmission in Iquitos, Peru, to evaluate the relationship between infection history and disease outcome. Iquitos has been the site of intense DENV transmission since the early 1990s, with large outbreaks due to DENV-1, DENV-2 and DENV-3 since then. In 2008, DENV-4 was introduced into the city and became the dominant serotype over the 2008/9 and 2009/10 transmission seasons (>99% of all cases). During that time, 1,397 participants in the longitudinal cohort seroconverted to DENV-4 (35% of the study population). Of these, 5.7% experienced a clinical case of dengue fever as detected by active door-to-door febrile surveillance. No effect of pre-existing antibodies against heterologous DENV serotypes was observed on DENV-4 seroconversion rates. However, we observed that primary and secondary infections resulted in a higher rate of symptomatic illness (8.7% and 9.0%, respectively) than third infections and fourth infections (3.1% and 3.3%, respectively). These data suggest that although pre-existing antibodies did not confer sterile immunity to heterologous serotypes, there may have been a cumulative protective effect against symptomatic illness. Further analysis is being conducted on the effects of specific serotypes, levels of neutralizing antibodies and time interval between infections.

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CIRCULATION OF DIFFERENT LINEAGES OF DENV-2 IN GUATEMALA DURING RECENT DENGUE EPIDEMICS, THEIR EVOLUTIONARY TIME-SCALE AND SELECTION PRESSURE ANALYSIS

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Dengue is the most common arboviral disease worldwide. Dengue is caused by dengue virus (DENV), which exist in nature as a complex of four different viruses or serotypes (DENV-1 to 4), belonging to the genus Flavivirus. DENV serotypes have been classified into different genotypes based on phylogenetic analysis from sequences of different viral regions. Dengue is endemic in Central America and is present in Guatemala since at least 1978. Here we report the phylogenetic relationships of the first fully sequenced DENV-2 from Guatemala (GU/FDA-GUA09/2009) with strains representing all known DENV-2 genotypes. Phylogenetic analysis of the envelope (E) protein and whole coding region sequences by maximum-likelihood and Bayesian inference methods revealed that at least two lineages of the American/Asian genotype of DENV-2 have circulated in that country during the 2007 and 2009 epidemics, and have possibly co-circulated during these and other epidemic periods. We found that the time to most recent common ancestor for Central

American DENV-2 of American/Asian genotype existed about 18 years ago, and Bayesian Skyline analysis revealed that the genetic diversity of this DENV-2 genotype in the region has increased since 2005. Site-specific selection pressure analysis revealed positive selection in the NS2A, 4B and 5 proteins but none in E protein. The study of dengue evolution in endemic regions is of importance, since nucleic acid technology (NAT) assays has been developed and implemented in the detection of DENV in these countries, as well as in places in which the disease is imported and can cause autochthonous transmission, as it happened recently in the US. Even though primers and probes for these assays are designed to target the most conserved regions of the viral genome, there is always a risk that the assay could fail to detect variants with mutations located at the target area. Therefore, the use of whole genomic sequences in molecular epidemiology studies appears to be more suitable for identifying mutations that may occur throughout the viral genome, some of which could potentially impact the performance of detection assays.

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EVALUATION OF LOW DOSE MONOVALENT DENGUE VACCINES IN HUMAN VOLUNTEERS

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Infections caused by the four serotypes of dengue virus represent a substantial burden of vector-borne disease. Globally, 3.6 billion persons are at risk for dengue infection; outcomes range from a self-limited febrile illness to a fatal shock syndrome. As part of the NIH dengue vaccine development program, we performed phase I clinical trials on two live attenuated monovalent dengue vaccines, DEN1Δ30 and DEN2/4Δ30, at a dose of 10^1 PFU to further evaluate the safety profile and to determine the human infectious dose 50% (HID_{50}) of these candidates. Results were compared to prior studies at higher doses of each vaccine (10^3 PFU). Flavivirus-naïve healthy adult volunteers were dosed with 10^1 PFU of DEN1Δ30 or DEN2/4Δ30 (15 each) or placebo (3 each) and followed for 6 weeks. Subjects were screened for viremia for 16 days and seroconversion was determined on days 28 and 42. For the two vaccines at lower dosages relative to the higher dosages, no significant differences were observed for headaches, myalgias, arthralgias, rash, or neutropenia. Nearly identical seroconversion levels were achieved for DEN1Δ30 at both doses: 93% for 10^1 PFU and 95% for 10^3 PFU. However, peak geometric mean titers were lower (91.5 vs. 160.6, p=0.029) at 10^1 PFU. Transient viremia was similar for DEN1Δ30: 8 (53%) volunteers for 10^1 PFU and 9 (45%) for 10^3 PFU. The duration of viremia was the same (2.8 days), but the mean day of onset was delayed for the low dose cohort (D12 vs. D 10). In contrast, for DEN2/4Δ30, only 53 vs. 100% seroconverted at the 10^1 vs. 10^3 dose (18.6 vs. 120, p=0.001 Peak GMT), though fewer vaccinees were viremic (33 vs. 55%). Viremia onset was delayed for the low dose (D13 vs. D9)s, but again the duration was similar (3.5 vs. 3.2 days). These data suggest that the human infectious dose (HID_{50}) of the DEN1Δ30 candidate vaccine is $\leq 10^1$ PFU, however that of rDEN2/4Δ30 is approximately 10 PFU, indicating lower infectivity of this candidate. These data suggest a higher dose of DEN2/4Δ30, relative to other components, may be required in a tetravalent formulation.

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PHYLODYNAMICS AND CHARACTERIZATION OF NATURAL ATTENUATION IN A SOUTH PACIFIC DENV-2 OUTBREAK

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Dengue is an arboviral disease that has seen a recent increase in activity throughout the tropics in recent decades, marked by more frequent and severe epidemics. While the causes of this reemergence are probably multifold, including geographic expansion of both vectors and viruses, the importance of virus strains with greater fitness, epidemic potential and possibly virulence, has been implicated. This prompted us to investigate the role of virus molecular evolution in driving epidemics. Our study was a series of outbreaks of American genotype DENV-2 in the South Pacific beginning in 1971 in Tahiti and Fiji, which became increasingly severe in New Caledonia and Niue Island in 1972. In Tonga in 1974, however, it became dramatically attenuated, with near-silent transmission. To elucidate the relative contribution of viral genetic change in outbreak dynamics, we conducted whole-genome phylogenetic analysis of DENV-2 strains collected during the South Pacific sweep paired with *in vitro* assays of comparative viral infection phenotype. Because all islands were equally immunologically naive for dengue, this study offers an opportunity to isolate the effects of viral genetic variation from differential herd immunity on epidemic behavior. We studied 17 low-passage DENV-2 strains isolated during outbreaks on the islands of Fiji, Tahiti, New Caledonia, American Samoa and Tonga. Each isolate was subjected to whole genome sequencing and phylogenetic analysis then compared for infection efficiency, replication rate and productivity in cell culture. We found variations in the coding portion of the dengue genome, particularly the pre-membrane gene (prM) and the nonstructural genes, NS2A and NS4A that correlate with the attenuation of the Tongan strains of virus. Phenotypic characterization of viruses bearing these genetic substitutions, in terms of their potential to account for different epidemic dynamics, will be discussed. Our analysis indicates a significant role for viral genotypic change in dengue epidemic severity.

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DENGUE VIRUS INFECTION MODULATES EXPRESSION OF REGULATORY COMPLEMENT FACTORS IN HEPG2 CELLS

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Several organisms, including flaviviruses, exploit the regulatory mechanisms of the complement system to evade innate immune responses. Elevated activation of complement system is present in severe dengue cases. Hepatocytes express several soluble complement factors and receptors; and liver damage is often found in severe dengue patients. CD46 and CD55 are cellular regulatory proteins that inhibit complement activation on the host cell surface and protect healthy cells from inflammation, whereas gC1qR is a C1q receptor that has been shown to be involved in modulating cellular anti-viral responses by disrupting MDA5 and RIG-I signaling. We hypothesized that dengue virus (DENV) exploits the complement regulatory mechanism to evade direct attack of the complement system compromising the homeostatic control of complement activation. Thus, we investigated the regulation of CD46, CD55 and gC1qR and complement deposition in a hepatoma cell line HepG2 infected with DENV serotypes 2 (Thailand 16681) or 3 (H87). The cells were analyzed both for virus infection and for the expression of above-mentioned receptors by flow cytometry. The frequency of infected cells (DENVpos) was approximately 50% for both viruses. The expression of complement receptors in DENVpos and bystander cells (DENVneg)

revealed that, regardless of the serotype, the expression of CD46, CD55 and gC1qR on DENVpos cells was increased, whereas on DENVneg cells the expression was decreased. Consistent with this finding, we observed an increased deposition of complement factors, iC3b and C6, on cells with lower expression of CD46, CD55 and gC1qR. These findings suggest that DENVpos cells are protected from direct complement attack, whereas DENVneg cells are more susceptible. Overall, these results reveal a mechanism to allow virus replication in DENVpos cells by protecting them against complement attack and inhibiting the intracellular factors for virus detection and suggest an indirect effect of virus infection on bystander DENVneg cells making them more vulnerable to inflammation and liver damage.

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DIFFERENTIATING THE EFFECTS OF DENGUE VIRUS INFECTION AND *AEDES AEGYPTI* SALIVARY PROTEINS IN DENDRITIC CELL IMMUNITY

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Hematophagous arthropod saliva has been shown to possess a variety of effector functions that facilitate the acquisition of a blood meal. Mosquito saliva contains molecules with anti-inflammatory, anti-hemostatic, and immuno-modulatory capabilities. Arbovirus-infected mosquitoes expectorate saliva and virus immediately prior to blood feeding and this saliva may have the potential to aid the establishment of arbovirus infection within the vertebrate host. Dengue virus (DENV), a mosquito-borne flavivirus, is also known to modulate various components of the immune response to infection. As such, it is necessary to differentiate the immuno-modulatory effects of the virus from those of the mosquito and to examine their possible synergism. The effects of *Aedes aegypti* saliva on the immune response profile of monocyte-derived dendritic cells were examined using multiplex cytokine immunoassays and ELISA. To reduce the variance between samples often observed in immunological studies of multiple donors, the human monocytic leukemia cell line THP-1 was used in place of primary human peripheral blood mononuclear cells. Treatment with DENV type 2, strain 16803, results in down-regulation of numerous cytokines involved in the innate immune response as well as those that would shift the response toward Th2. Treatments with saliva and virus each raised the secretion levels of TNF-alpha and IL-8, and the combined effects of treatment with saliva and virus further increased expression. Additionally, IL-1beta was significantly up-regulated in treatments including saliva while virus alone had no such effect. The continued characterization of both the isolated and synergistic effects of saliva and virus is vital to the understanding of the immunological environment during infection establishment as well as developing possible therapeutic applications.

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HYPERENDEMIC TRANSMISSION OF DENGUE IN NORTE DE SANTANDER, COLOMBIA

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Dengue virus is a significant international public health threat with the potential to become a health security issue as it continues to emerge throughout the tropics and reaches across national borders. The incidence of dengue infections in people is increasing within endemic regions across the globe. Coincident with this emergence, dengue is also expanding into less recently afflicted areas with increasing effect (including autochthonous transmission establishment). Currently the department of Norte de Santander, Colombia is experiencing the highest rate of DENV cases in the country. Specifically, in Cucuta and Los Patios, transmission has intensified. In cooperation with local hospitals and the health department of Norte de Santander, we tested serum collected from suspected dengue cases. It was believed that dengue 3 was absent from the area, largely based on the incidence of this serotype in the neighboring Venezuela. We show that not only is D3 circulating and infecting people in Norte de Santander, but it is at a comparable rate as dengue 1 and 2, speaking to the continuing expansion of the virus. Dengue 4 is largely overshadowed by the other serotypes, though this report confirms the co-circulation of all four serotypes in this area. Of particular interest was the presence of four double positive individuals. Three individuals in Los Patios (D1/D2, D1/D3, D1/D2) and one individual in Cucuta (D2/D3) tested positive for two serotypes. Preliminary sequencing shows that D1 and D3 are genotypes that have been circulating in Colombia since 2008. D2, however, has 100% homology with a Venezuelan strain isolated in 2007. Further sequencing will inform phylogenetic relationships and possibilities of temporal and spatial patterns of viral movement. The presence of all four serotypes indicates this is a hyperendemic area with high levels of transmission. These interactions require further investigation to further inform public health officials on dengue transmission in this region.

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SEASONAL PATTERNS OF DENGUE VIRUS TRANSMISSION IN IQUITOS, PERU

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Understanding periodicity in disease dynamics is fundamental to predicting outbreaks and reveals factors involved in disease etiology. Dengue virus (DENV) has been shown to exhibit annual and multi-annual patterns of transmission, presumably driven by climate. We examine ten years of laboratory-confirmed, acute DENV infections captured by passive, clinic-based surveillance and routine entomological monitoring of adult *Aedes aegypti* population densities in Iquitos, Peru where there is minimal climatic variation (daily temperature = 25.9ºC ± 2.2). Wavelet analysis of weekly DENV cases indicates a strong, annual signal of increased transmission and weak evidence of periodicity on a three-year scale. The number of cases peaks annually in December, is lowest in July, and is strongly correlated with mean daily temperatures, which consistently dip in early July. As has been observed in Thailand, however, mean temperatures do not significantly differ between high transmission and low transmission seasons (26.9ºC vs 25.6ºC), but neither does the daily temperature range (10.3ºC vs 10.3ºC). Thus it appears that temperature variation is insufficient to explain annual periodicity in Iquitos, because although the short period of low temperatures in July could effectively slow viral replication in mosquitoes, transmission rates decline well before that time (~March) and do not elevate until well after (~October). Furthermore, precipitation decreases during June and July (0.25 inches/day, annual mean 0.38 inches/day), but otherwise is constant throughout the year, and variation in *Ae. aegypti* adult population densities does not show any consistent pattern that fluctuates with trends in transmission. Viral fade out in the human population appears to be strongly influenced by near-annual fumigation campaigns organized to control DENV outbreaks, while annual amplification in the population remains unexplained. We present and discuss a number of alternative models explaining annual amplification as well as inter-annual variation in the shape and magnitude of epidemic curves.

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ENVIRONMENTAL AND AGING INFLUENCES ON ANTIBODY-ENHANCED DENGUE DISEASE OUTCOMES IN AN IMMUNOCOMPETENT MURINE MODEL

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T-lymphocytes are proposed to promote clearance during primary dengue virus (DENV) infection but contribute to immunopathology during heterologous infections. Since an enriched environment enhances T-cell activity during viral infections and active older adults show less functional decline in T cell adaptive immunity, we hypothesized that enriched environment and aging would increase disease severity. To induce multiple infections of a single serotype or antibody-enhanced disease as it may occur in human infection, serial i.p. injections with DENV3 (genotype III) infected brain homogenate or anti-DENV2 hyperimmune serum followed 24h later by DENV3 (genotype III) infected brain homogenate were done. Control mice received anti-DENV2 hyperimmune serum followed 24h later by uninfected brain homogenate. Compared to antibody-enhanced dengue disease, clinical signs after one serotype infection were less apparent and survival periods longer. After antibody-enhanced dengue disease significant differences in the survival probability curves ($p = 0.031$) were found and both young and aged subjects from enriched environment showed higher mortality, intense clinical signs and hyperplasia of T cells in liver and lungs than subjects with impoverished environment. We propose that an enhanced immune response is occurring in the subjects of the enriched environment and in line with this concept glucocorticoids reduced these adverse outcomes

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LONGITUDINAL CHARACTERIZATION OF ANTIBODY RESPONSE TO DENGUE VIRUS IN BANGKOK THAILAND

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The plaque reduction neutralization test (PRNT) is the gold standard used to characterize the serologic immune response during and after infection with dengue virus (DENV). Few studies have described the trajectory of PRNT titers to all four serotypes after infection. We illustrate the antibody response to infection in a cohort of Thai children through parallel analysis of longitudinal PRNT results for all four serotypes of DENV. One hundred and eighty children from 1 to 15 years old seen at 2 hospitals in Thailand with RT-PCR confirmed DENV infection were followed up for dengue virus antibody response. Blood samples were collected daily from enrollment to the day after defervescence. Subsequent samples were collected at one week, six months, and yearly, with a maximum of three years of follow-up. Children were categorized based on their first PRNT measurement as undetectable ($PRNT_{50} < 10$ to all DENV serotypes), monotypic ($PRNT_{50} > 10$ to only 1 DENV serotype) or, multitypic ($PRNT_{50} > 10$ to more than 1 DENV serotype). We modeled the mean rate of ascent, time to peak, and rate of decline for children in all three initial PRNT categories. We observed a consistent response across all serotypes characterized by an approximately linear rise in log titer followed by a peak 5-10 days after return to normal body temperatures. Through analysis of the complete PRNT trajectory of those with a secondary infection, we find evidence that the serotype with the highest titer at first sample dominates the secondary neutralizing response thus supporting the theory of original antigenic sin. Furthermore, we find a rise in titer trajectories beginning two years after secondary infection that may be explained by subclinical tertiary or quaternary infections. These results help characterize the longitudinal serologic immune response to infection with dengue virus and can assist in the interpretation of PRNT results taken at different times from infection.

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WHAT DO PEOPLE KNOW AND DO ABOUT DENGUE AND PROTECTING THEMSELVES FROM IT IN IQUITOS, PERU?

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As part of a community-randomized trial to evaluate the effectiveness of insecticide-treated curtains (ITC) for dengue prevention in Iquitos, Peru, we applied a survey between October-December 2009 to 1334 study participants to examine their knowledge, attitudes and practices (KAP) associated with dengue and its prevention. Most of our respondents were female (73.9%), had finished secondary school (78.4%), and had a median age of 39 (range: 16-88). Although most participants knew that dengue was transmitted by a mosquito bite (85%), only 16.5% recognized that this mosquito bites during the daytime, 19.3% knew that its legs have white stripes, and 14.7% knew dengue is transmitted by *Aedes aegypti*. The most commonly recognized symptoms of dengue were fever (86.5%), headache (76.4%), muscle or joint pain (57.2%), and nausea or vomiting (25.0%). The most commonly identified preventive practices included getting rid of unusable items that might collect water (37.3%), and use of products to kill or repel mosquitoes (13.5%). More than half the respondents knew someone who had had dengue at some point (65%), and amongst these individuals, the median number of people they knew was 2. When people were asked what one should do if one has dengue, only about half (54.1%) knew to take paracetamol. Most common practices for mosquito control people mentioned were cleaning their homes (46.9%), picking up unusable items that might collect water (37.3%), covering water containers (26.2%), fumigating their homes (17.8%) and using insecticides around their home (13.5%). Use of insect repellent was minimal (2%). Despite (1) dengue endemicity in Iquitos for the past decades, (2) that the Regional Health Authority routinely fumigates and places larvicide in water containers, and (3) that there have been different types of health education messages at the community level disseminated through various media (radio, signs on buses and other public places), knowledge about dengue and its transmission, as well as household level practices to reduce dengue, could be improved in Iquitos.

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LEPTOSPIROSIS AND DENGUE FEVER CO-INFECTION: A REPORT OF THREE REPRESENTATIVE CASES

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Leptospirosis and dengue fever cause overlapping symptom profiles leading to mis-diagnosis, higher morbidity and mortality. Concurrent leptospirosis and dengue infections have not been widely studied. We report 3 representative cases of co-infection during the 2008 dengue epidemic in American Samoa. Hospital infection control records from

January to September were analyzed for dengue and leptospirosis co-infection cases. Three cases representing a spectrum of disease and treatment in outpatient, inpatient and critical care settings were identified. Patient medical records were reviewed retrospectively. Data included demographic information, history and physical exam findings, laboratory and imaging results, treatment, length of hospital stay, severity of illness, complications and final outcomes. Of the 132 dengue IgM+ patients, and 17 leptospirosis + patients identified during the study period, six were identified to have a co-infection. Representative cases demonstrate disease, treatment, and hospital setting variability. Case one is of a 41 year-old male presenting to the emergency department (ED) with fever, chills, and headache. Laboratory analysis during two outpatient visits demonstrated leucopenia, hemo-concentration, thrombocytopenia, elevated lactate dehydrogenase (LDH) and creatinine phosphokinase (CPK). Concomitant lower extremity cellulitis complicated decision making. Symptoms resolved with supportive outpatient care. Case two is of a 33 year-old female presenting to the ED with chills, dizziness and nausea. Initial laboratory assessment demonstrated leucopenia, hemo-concentration, thrombocytopenia, and mild AST and ALT elevations. On day two she demonstrated a fine erythematous rash, critical thrombocytopenia, alkaline phosphatase (ALP), and LDH elevations. Critical thrombocytopenia resolved with two days of supportive inpatient care. Case three is of 32 year-old female evaluated in the ED and admitted to the Critical Care Unit for septicemia and multi-system organ failure. Critical thrombocytopenia developed with resultant hemorrhagic disease and eventual death. Comparative case descriptions, laboratory analysis and treatment reviews are provided. In conclusion, challenges identifying dengue fever-leptospirosis co-infections result in treatment delays and adverse outcomes. Dengue epidemics in American Samoa may require routine testing for both diseases.

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SEA SURFACE TEMPERATURE MONITORING FOR DENGUE EARLY WARNING IN ECUADOR

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Dengue fever, a mosquito-borne viral disease, is one of the most important emerging tropical diseases in Ecuador. We report a statistical model for assessing the importance of climate as a driver for inter-annual variability in dengue fever in southern coastal Ecuador. Climate variables from a local meteorology station (precipitation, relative humidity, min/max/mean air temperature) and Pacific sea surface temperature (SST) anomalies were used to predict annual dengue fever incidence (1993-2010). Non-climate confounding factors such as serotype introduction were also considered. During El Niño events (positive Pacific SST anomalies), southern coastal Ecuador experiences warmer and wetter conditions, while during La Niña events (negative Pacific SST anomalies), the climate is cooler and drier. Preliminary results indicate that years with an above normal incidence of dengue fever were associated with El Niño events and years with below normal incidence of dengue were associated with La Niña events. Increased rainfall and warmer temperatures increase the availability of breeding sites and the development rate of the dengue mosquito (*Aedes aegypti*). Due to time lags involved in the climate-disease transmission system, monitoring El Niño / La Niña evolution in the Pacific Ocean could provide some predictive lead for forecasting dengue epidemics. This is the first study of dengue fever and climate in this region. This research provides the foundation to develop a climate-driven early warning system for dengue fever in Ecuador.

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ECO-EPIDEMIOLOGICAL EVALUATION OF CHAGAS DISEASE PREVALENCE IN THE VILLAGE OF LAGARTERA GRANDE, REPUBLIC OF PANAMA

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Chagas disease has historically been endemic to the village of Lagartera Grande, especially in children. Previous data collected in 2004 revealed that 2.9% of the village children tested seropositive for *Trypanosoma cruzi*, the parasite responsible for Chagas. Once infection was detected in this community, health officials began to implement preventive measures. The Gorgas Memorial Institute conducted health education projects in the community in cooperation with the Japan International Cooperation Agency (JICA) with particular focus on educating school children on various aspects of the disease. The Department of Vector Control of the Ministry of Health began sporadic spraying of infested houses and developed an efficient system of entomological surveillance with community participation. This study serves to evaluate the progress accomplished since 2004 by assessing the current prevalence of Chagas within Lagartera Grande. Seventy-seven members of the community completed the Knowledge, Attitudes and Practices (KAP) survey for Chagas disease, the vector *Rhodnius pallescens*, and the vector's arboreal habitat the royal palm *Attalea butyracea*. Serum samples were also collected from all the children in Lagartera Grande above the age of six months to assess the current prevalence of infection in the community. Samples were screened with 3 different serological tests: a commercial recombinant enzyme-linked ELISA (ELISA Chagatest, Wiener Laboratory, Argentina), a recombinant ImmunoComb commercial test (Orgenics, Israel), and an immunoblotting technique using a crude epimastigote antigen preparation derived from a Panamanian *T. cruzi* strain. All samples collected tested negative, suggesting the successful implementation of preventive measures in the community. Continued surveillance and monitoring was recommended since favorable conditions for transmission are still present in Lagartera Grande.

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IDENTIFICATION OF ANTHROPOLOGICAL AND SOCIOCULTURAL RISK FACTORS FOR CUTANEOUS LEISHMANIASIS IN CAPIRA DISTRICT, PANAMA

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Leishmaniasis is a disease with multiple clinical presentations that affects millions of individuals around the world. This parasitic disease is complex and difficult to control due to the intricacies of its transmission cycle, the variety of animal host reservoirs, the many species of sand flies that act as transmission vectors, and diversity of ecosystems involved. In Panama, there is an increasing incidence of the ulcerated cutaneous form of the disease and it is suspected that there is an underestimate of the actual number of people affected due to lack of access to healthcare by poor and disadvantaged populations. This project aimed at identifying risk factors for cutaneous leishmaniasis that can be useful in establishing a prevention plan tailored to the community where cutaneous lesihmaniasis is endemic. The population studied was from the village of Trinidad Las Minas in Capira District, Republic of Panama. One hundred and twenty-five individuals older than 12 years of age were surveyed between November and December 2009 in the village. Twenty-four households were randomly chosen and the characteristics of each household were recorded to identify risk factors. The variables studied included the floor material,

the roof material, the presence of cracks on the walls, the presence of surrounding royal palms, the presence of other animals around the home, and the presence of screens on the windows. The data was analyzed using descriptive statistics and univariate logistic regression. On univariate analysis the only variable associated with cutaneous leishmaniasis in the home was the presence of dirt floors (χ^2 = 6.8, P = 0.01). Multiple logistic regression will be carried out to determine whether this is an independent factor. In conclusion, the floor material should be further studied as a potential risk factor for cutaneous leishmaniasis. The results may be useful in developing a community prevention plan with the goal to decrease the incidence of the disease.

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SUDANESE *LEISHMANIA DONOVANI* POPULATION STRUCTURE: UNCOVERED BY MULTILOCUS MICROSATELLITE AND SEQUENCE TYPING

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The population structure of *Leishmania donovani*, the etiological agent of visceral leishmaniasis (VL), was investigated. VL is highly endemic in countries such as India, Brazil and Sudan. Sudan has suffered serious epidemics in the past, particularly in the East and South. Here we had typed a large panel of Sudanese strains using multilocus microsatellite typing (MLMT) (n=14 markers, 103 isolates) and multilocus sequence typing (MLST) (n=11 targets, 50 isolates). Both genetic typing methods agreed on the presence of two main groups of L. donovani in Sudan: group R which contained 3 subpopulations as estimated by MLMT (SDA, SDB and SDD) or 2 subpopulations as estimated by MLST (SDA and SDB+SDD) and group G (~1:1 canine: human isolates) which contained 2 subpopulation (SDC and SDC-outliers) as suggested by both typing methods. All subpopulations showed a significant deficiency of heterozygosity that cannot be explained by a Wahlund effect except SDA which showed higher than expected heterozygosity for MLMT markers only. In addition, subpopulation SDB of MLMT Group R was not significantly departed from Hardy-Weinberg equilibrium, was in linkage equilibrium and had an inbreeding index (FIS= 0.20), selfing rate (s=0.33) and panmictic index (f= 0.02) values compatible with recombination. In this study, it is tempting to suggest that this subpopulation is an intermediate between SDA and SDD, but this hypothesis needs to be further investigated. Furthermore, all MLST subpopulations showed none to minimal pairwise linkage disequilibrium after sequential Bonferroni correction (α= 0.05). The equal number of isolates from dogs and humans in group G suggests a role in transmission for domestic dogs, at least in eastern Sudan. However, subpopulations SDC and SDC-outliers were moderately subdivided to effectively isolated from Group R subpopulations (FST values ≥ 0.40). An association was suggested between the subpopulation and pathology, which if holds true after further investigations, may be very useful for disease diagnosis and pathology-specific designed drugs. This study provided a plethora of new information regarding the population genetics of Sudanese L. donovani, and raised several hypothesis regarding important aspects of the epidemiology of leishmaniasis in Sudan, and possibly other endemic areas.

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MULTIPLE ETIOLOGIC AGENTS; THE POSSIBLE CAUSE OF CUTANEOUS LEISHMANIASIS IN GHANA

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The emerging and/or re-emerging focus of cutaneous leishmaniasis (CL) in Ghana since 1999 has in recent times, seen more than one species of the parasite identified and implicated as the etiologic agent. Leishmania *major* was first to be identified as the agent of the infection in 2006 in the endemic focus in Ghana. An unknown and uncharacterised *Leishmania* species was identified in 2007 but did not use species linked to African leishmaniasis as positive control. The recent work done in 2008 using *Leishmania* species associated with infections in Africa as positive controls identified *Leishmania* contrary to the species previously recognized, as one of the possible species influencing disease in Ghana. This study aimed at the identification of species of *Leishmania* parasites responsible for CL focus reported in Ghana. The endemic focus is located in the south-eastern part of Ghana, which borders three countries in the West African sub-region. Twenty lesion aspirate and scraping samples were taken from active patents lesions for the study. Primers A1 and A2, were used to amplify a fragment of ~1500 bp of the intergenic region between the ribosomal protein genes RPS7A and RPS7B on chromosome 1 and second primers B1 and B2, were used to amplify an internal fragment of ~1350bp in a nested PCR. These nested PCR products obtained were digested using restriction enzyme *MspI* and the products run on 2% agarose gel. The bands produced from some samples showed a match to one of the control sample *L. aethiopica*, hitherto is found to be associated with leishmaniasis in the eastern part of Africa. Comparing this preliminary results to previous works by other investigators, one can somewhat say that there could be more than one agent responsible for CL focus in Ghana.

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TRYPANOSOMA SPP. OF RODENTS AND OPOSSUMS IN A CHAGAS DISEASE ENDEMIC REGION OF NORTHERN PERU

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An estimated 192,000 Peruvians are infected with *Trypanosoma cruzi*, the etiologic agent of Chagas disease. In an endemic region of northern Peru in the province of Cutervo, intervention methods, including spraying, began in 2001 that seemingly decreased the incidence of Chagas disease by the next year. Despite these efforts, new acute cases (2 months- 2 yrs) were reported from 2004-2008 and prevalence from a 2004 study was still 27.7% in individuals <15 years of age. Many communities in Cutervo are in close contact with the sylvatic environment having mud brick homes in the middle of the Andean high jungle ecosystem. Considering the presence of new cases and the sylvatic environment, the goal of the current study was to determine the role of wildlife reservoirs in *T. cruzi* transmission within the region. Rodents (*Rattus norvegicus* and *R. rattus*) and opossums (*Didelphis albiventris*) were live captured from within homes and surrounding areas in five communities (Casa Blanca, Pindoc, Esperanza, Rumiaco, and Nuevo Guayaquil). At the time of collection, trypomastigotes were observed by bright-field microscopy in 22 of 50 rodent whole blood samples, while no parasites were observed in opossums (n=7). A panel of PCR, including gene targets for the minicircle

and 28S, 18S, and region from 18S to 5.8S rDNA, was run to identify false negatives by microscopy and determine the *Trypanosoma* spp. present. Of the rodents, 16/22 positive by microscopy were infected with *T. lewisi*, while the *Trypanosoma* spp. of the remaining six rodents could not be identified due to sample loss. *T. cruzi* was identified in 4/7 opossums; these animals were hand-captured south of the Esperanza community. While the sample size of the current surveillance study is small, two main observations were made: 1) Rats do not appear to play a role in the *T. cruzi* transmission cycle within Cutervo and 2) the opossum may have a potential role as a wildlife reservoir for *T. cruzi* in the area.

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EPIDEMIOLOGICAL CHARACTERISTICS OF LEISHMANIASIS IN PERU 2004-11

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Leishmaniasis is a parasitic disease of significant public health importance. Considered by WHO as one major Neglected disease, this disease is Transmitted by sand fly vector. In Peru, leishmaniasis is an endemic disease affecting several departments and is the second endemic tropical. Is reported an annual average of 2500 cases. There is a need to provide information for the management of the disease. The study Focus on the determinate characteristics epidemiological and distribution of cases. Notified leishmaniasis is near mandatory public health services. All suspected cases were recorded in the Epidemiological sheet and reported to the epidemiological surveillance system in the country. From 2004 until 2010, were reported 19113 cases of leishmania. Of all cases 92.6% were cutaneous leishmaniasis and the remaining 7.4% mucocutaneous leishmaniasis. Most have come from Cusco with 3318 cases (16.1%), Ancash with 1836 cases (8.9%), Piura with 1816 cases (8.8%), Junín with 1797 cases (8.7%) and San Martin with 1711 cases (8.3 %). Until week epidemiology 13-2011, 1471 cases have been reported, which makes a national incidence rate is 101.6 per 100 000 inhabitants. The endemic area extends through the Andes and the valleys between 600 and 3 000 meters above sea level, for cutaneous leishmaniasis, and areas of high and lowland forest below 2000 meters for mucocutaneous leishmaniasis. The age group was most common entre 20 and 49 years old (67.56%). The male / female ratio was 1.6. The majority of patients were farmers. The unique lesion was The Most Frequent (69.8%) and the Majority of injuries was in extremities (58.2%). In conclusion, leishmaniasis is endemic in tropical kind in Peru and is distributed in poor areas of various departments. This produces a negative social and economic impact in the economically depressed. In addition, the destructive consequences it causes, particularly the mucocutaneous form the effect of isolating the individual, its irreversibility.

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CAN WE REDUCE PERSONAL RISK OF RHODESIAN SLEEPING SICKNESS? ANALYSIS OF FACTORS CONTRIBUTING TO THE PROBABILITY OF BEING BITTEN BY TSETSE

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Rhodesian sleeping sickness, the zoonotic form of Human African Trypanosomiasis found in east and southern Africa, is often associated with game parks and wilderness areas where tsetse flies (*Glossina* spp.) and wild reservoir hosts are abundant. People living and working within or near such areas have limited options for controlling HAT due to concerns about the cost, feasibility and environmental impacts of tsetse control. In the Mana Pools National Park of Zimbabwe, we carried out studies to identify the circumstances in which people are most likely to be bitten by infective tsetse. Our results show that, contrary to expectation, people were more likely to be bitten by tsetse (*G. morsitans morsitans*, *G. pallidipes*) in the vicinity of their houses rather than in the woodlands (total catches = 375 *vs*. 264 tsetse caught over 221 days) where tsetse are apparently more abundant. For tsetse from houses, 44% were female and of these, 30% were old enough to be able to carry mature infections. Moreover, natural repellents (human body odour, woodsmoke) produced by humans which are highly effective outdoors, are ineffective against indoor-biting tsetse. For humans in woodlands, the numbers of tsetse were greatest if the human was mobile and not in the vicinity of a natural non-human host: humans walking without an ox caught 20 tsetse/day compared to 0.2 tsetse/day for a stationary human accompanied by an ox, albeit the flies were younger and hence less likely to be infected (18% of females were old enough to be able to carry mature infections). We suggest that for people in areas where Rhodesian sleeping sickness poses a risk, interventions designed to prevent or kill tsetse entering houses or vehicles might reduce personal risk of HAT significantly.

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POPULATION STRUCTURE OF *LEISHMANIA TROPICA* IN NORTHERN PAKISTAN AND NEIGHBORING COUNTRIES

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In Pakistan, Anthroponotic Cutaneous Leishmaniasis (ACL) caused by *Leishmania tropica*, has a broad distribution occurring focally in the Northern areas and Azad Kashmir. Reportedly, some isolates of *L.tropica* are heterozygous. This project aims to investigate the intra-specific diversity of *L.tropica* in Northern Pakistan using Multilocus microsatellite typing (MLMT). Further, the population structure and phylogenetics of the parasite will be mapped by taking into account isolates from Pakistan and from other countries lying between the Mediterranean Sea and the Bay of Bengal, namely Syria, Afghanistan, Iran and India. As a tool to enhance our ability to identify *L. tropica* from *L. major*, which is sympatric in this region, we have developed a novel PCR that distinguishes between these two species. Samples have been collected from 3 major hospitals of Peshawar, Khyber Pukhtoon-Khwa (KPK),Northern Pakistan. These include isolates in culture, biopsies and filter paper impressions. These samples have been typed for species and followed by MLMT for *L. tropica* isolates. MLMT analysis of clinical isolates from Pakistan and other countries in the region (confirmed as *L. tropica*), plus the WHO strains will be presented. Results providing an estimate of the ACL presentation rate at Peshawar hospitals will also be discussed briefly.

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TRANSMISSION DYNAMICS OF *TRYPANOSOMA CRUZI* LINEAGE I IN TWO ENDEMIC PROVINCES OF ECUADOR

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Trypanosoma cruzi infection affects an estimated 230,000 people in Ecuador. Recent reports indicate limited effectiveness of insecticide-based vector control interventions, due to re-infestation by sylvatic triatomines. Previous studies demonstrated that the lineage I (TcI) of *T. cruzi* is the predominant lineage circulating in Loja (Southern Andes) and

Manabi (Central Coastal) provinces. Furthermore, in southern Ecuador (Loja province) microsatellite analyses of TcI isolates showed two main parasite populations exist: one related with domestic and peridomestic environments and a second one related with sylvatic environments. The aim of this study was to evaluate TcI isolate divergence within and among Loja and Manabí using Multi Locus Sequence Typing (MLST). We sampled vectors and mammals and a wide geographic area within each province. Our results corroborate that the presence of two different parasite populations in Ecuador, according to habitat: In Loja province, the previous separation in two populations (domestic/peridomestic and sylvatic) was confirmed. In Manabí province, this tendency was also seen, where the sylvatic population was separated from the peridomesic population. However, in both cases, limited genetic flow was evidenced. Interestingly sylvatic samples of both provinces cluster together, suggesting genetic flow among sylvatic populations, while genetic separation was evident between domestic/peridomestic populations of both provinces. These results suggest that similar transmission dynamics are taking place in both provinces where albeit at different rates, there is limited genetic flow between sylvatic and domestic/peridomestic *T. cruzi* populations within a small geographical area. Therefore, control strategies need to be adapted to the intrinsic characteristics of a small geographic scale.

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DISTRIBUTION AND NATURAL INFECTION OF CHAGAS DISEASE VECTORS IN DOMESTIC, PERIDOMESTIC AND SYLVATIC HABITATS IN SOUTHERN ECUADOR

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Chagas disease is endemic in 70% of the Ecuadorian territory. The main vectors responsible for *Trypanosoma cruzi* transmission in the Southern Andean region of the country are *Rhodnius ecuadoriensis, Triatoma carrioni, Panstrongylus chinai* and *P. rufotuberculatus.* This study aims to describe the triatomine distribution and natural trypanosome infection in domestic, peridomestic and sylvatic habitats in Loja Province. Active triatomine searches were conducted in domestic and peridomestic habitats in rural villages and in nearby sylvatic areas throughout the province. 11,115 live triatomines were found infesting domestic units in 68% of the 92 rural communities while 1,923 live triatomines were found in 52% of the 23 sylvatic localities examined. Nine percent of the domestic units (n = 3,191) were infested with one or more triatomine species and 12% of the sylvatic nests (n=1,219) were infested with *R. ecuadoriensis*. Nymphs were observed in 80% of both infested domiciles and nests. Triatomines were found in all ecological regions below 2,200 meters above sea level. In the domicile *R. ecuadoriensis* and *T. carrioni* were found mostly in bedrooms while in the peridomicile these species were abundant in chicken coops located near the house. Established colonies of *P. chinai* and *P. rufotuberculatus* were found restricted to the domicile. Sylvatic triatomines were found mainly in squirrel and mouse/rat nests, and to a lesser extent in bird nests. *T. cruzi* infection was found in 10% of the domestic/peridomestic triatomines (n=775) and 64.7% (n=300) of sylvatic triatomines analyzed. Mixed infections with *T. cruzi* and *T. rangeli* were found in 8% of sylvatic triatomines. To date, limited vector control efforts have been implemented in this area. Although, the application of insecticide-based vector control could be effective in reducing domestic and peridomestic populations it must be complemented with constant surveillance to detect and control post-intervention reinfestation by sylvatic triatomines. Our findings highlight the need for a systematic, sustained, and monitored vector control intervention in the region.

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ANTI-TRIATOMINE SALIVA IMMUNOASSAYS FOR THE EVALUATION OF IMPREGNATED NETTING TRIALS AGAINST CHAGAS DISEASE TRANSMISSION

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Insecticide-impregnated nets can kill triatomine bugs, but it remains unclear whether they can protect against Chagas disease transmission. In a field trial in Quequeña, Peru, sentinel guinea pigs placed into intervention enclosures covered by deltamethrin-treated nets showed significantly lower antibody responses to saliva of *Triatoma infestans* compared to animals placed into pre-existing control enclosures. Our results strongly suggest that insecticide treated nets prevent triatomine bites and can thereby protect against infection with *Trypanosoma cruzi*. Anti-salivary immunoassays are powerful new tools to evaluate interventions against Chagas disease.

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PROXIMITY BETWEEN DOGS AND *TRYPANOSOMA CRUZI* INFECTED TRIATOMINES AS A RISK FACTOR FOR THE PERSISTENCE OF CHAGAS DISEASE

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Chagas disease is a vector-borne disease transmitted by triatomine bugs and caused by the *Trypanosoma cruzi* parasite. It is one of the most neglected tropical diseases. Insecticide application campaigns to eliminate vectors are the most effective intervention to stop transmission of the parasite, and are routinely conducted in Arequipa, Peru. After these campaigns, re-infestation can occur, and areas where vectors and *T. cruzi* infected mammals overlap can be the starting point for re-initiation of disease transmission. Dogs have been described as reservoirs of *T. cruzi* in Argentina, Brazil, Mexico, and Venezuela. Our objective was to determine whether the presence of seropositive dogs can explain the clustered re-emergence of *T. cruzi* in vectors. The study was designed as a cross-sectional serological screening to detect antibodies against *T. cruzi* in dogs, entomological collection of vectors from households to determine their infection status, and georeferencing of households to determine proximity between dogs and triatomines. The main outcome was canine seropositivity. Its association with other factors was analyzed with multivariate logistic regression for demographic and household risk factors and with spatial techniques for clustering and proximity correlation. Canine seroprevalence in the area was 12.3% (SE=2.6, N=154). The statistical results show that seropositivity in dogs was positively associated with proximity to *T. cruzi* infected triatomines, with proximity to high numbers of triatomines, regardless of their infection status, and with dog's age. The presence of *T. cruzi* seropositive dogs

could explain the persistence of Chagas disease in endemic areas of Peru. Massive insecticide campaigns allow for the collection of triatomine data that are used to determine high-risk areas for humans. Interventions based on these entomological data should include the presence of dogs around houses where infected triatomines were collected.

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GENETIC DIVERSITY OF *RHODNIUS ECUADORIENSIS* (*HEMIPTERA: REDUVIIDAE*) POPULATIONS IN THE CENTRAL AND SOUTHERN ANDEAN REGIONS OF ECUADOR

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Rhodnius ecuadoriensis is the most widespread vector of Chagas Disease in Ecuador. Effective control of this disease requires a good understanding of the epidemiological cycles, including a reliable analysis of the genetic structure of populations of this important vector. *R. ecuadoriensis* occupies domestic, peridomestic and sylvatic habitats and is a widely distributed species in the central Coastal (Manabí province) and southern Highlands (Loja province) regions of Ecuador. These two regions are phytogeographically and climatically different and correspondingly, bugs collected from these areas demonstrate differences in several phenotypic characters (i.e., body size, antennal sensilla and wing geometry morphometrics), as well as in behavioral traits (feeding and defecation patterns, and life cycle). To evaluate the genetic relationships among *R. ecuadoriensis* populations between these regions, we sequenced the mitochondrial cytochrome b (Cytb) gene in 168 insects collected from both regions (n=95 in Loja) and (n=73 in Manabí). We found 34 Cytb haplotypes determined by 53 variable sites. Only three haplotypes were shared between the two provinces (15 were exclusive for Loja and 16 for Manabí). A moderate genetic differentiation was observed between the two geographical regions (G_{ST}=0.05622) and remarkably, a third genetically different group within the Loja province was found. Our results support the hypothesis of disruptive selection acting upon *R. ecuadoriensis* populations, probably due to geographical isolation. The genetic patterns observed in this work contribute to the knowledge of genetic variability of *R. ecuadoriensis* across different geographical regions and provides background for interpretation of routes of dispersion and isolation.

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DEMOGRAPHIC AND SOCIOECONOMIC DETERMINANTS OF MOSQUITO NET USAGE IN MALARIA ENDEMIC REGION OF BANGLADESH

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Use of mosquito net is a proven strategy for malaria control. One of the vector control components of the National Malaria Control Program in Bangladesh was to promote insecticide treated bed net to ensure prevention or reduction of malaria mortality and morbidity. However, use of mosquito net as well as insecticide treated bed net varies among households. We investigate associated factors in bed net usage at Kuhalong and Rajbila unions under Bandarban Upazila, south-east region of Bangladesh. We utilized data of an ongoing demographic surveillance from 4567 households with population size 20755. Both the unions were divided into 12 clusters (C). Overall 99.2 % (4529) households possessed mosquito nets. Most cited reason for not using bed net while sleeping in 38 households was unavailability of a net. In Kuhalong and Rajbila respectively 89% and 87% of population slept under bed net at previous night of the interview whereas use of insecticide treated bed net was about 80% in both. Lowest percentage of last night bed net users were in C5 (63.5%) of Kuhalong and in C9 (80.2) of Rajbila. Insecticide treated net use was lowest in C11 (59.3%) and C4 (69%) of Kuhalong and Rajbila respectively. Person per bed net was as high as 3-4 in few areas of the unions. Nontribals, household heads, females, married persons, children <5 years of age, individuals from family size <5 were more likely to use bed net in previous night of the interview (P <0.001). Use of insecticide treated bednet was significantly higher among tribal counterpart and individuals from family size <5 (P<0.001). Last night bed net users were higher among persons from households that own radio/tape recorder (<0.001) or dwelling unit ≤2 (P <0.001). However, insecticide treated bed net users were also significantly higher among members of households having bamboo floor/wall (P <0.001) or using oil/kerosene lamp ((P <0.001). In both unions, there was no significant variation between malaria positives and last night bed net user. Gap between insecticide treated bed net user and nonuser should be reduced and more emphasis should be given to seek malaria associated risk factors in these areas.

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COMMUNITIES IN NETWORKS MAPPING MALARIA MOVEMENT IN KENYA

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With malaria eradication back on the global agenda and subsequent elimination targets for various low endemic countries, control strategies require a strong quantitative evidence base. The failure of previous elimination efforts has shown that human population movements are important for infection exchange between different transmission areas. For countries, that have overall low transmission but a few high transmission hotspots, population movements from high to low transmission zones may threaten imported infections, therefore local control agendas and challenge larger scale elimination efforts. Here, a unique and extensive mobile phone records dataset was analyzed with network analysis tools, a countrywide *Plasmodium falciparum* transmission map and previously developed transmission models to assess communities within Kenya linked by infection flows. The likely principle sources of imported infections within national boundaries, which may threaten onward transmission or have clinical significance, were mapped at a settlement level. Clusters of settlements were identified and compared to approximate "natural" malaria-relevant migration boundaries, splitting the country into regions that share malaria-relevant movement characteristics. With elimination as the ultimate goal for Kenya, we provide a quantitative platform for strategic control planning, by targeting control resources at defined spatial and temporal scales.

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SOCIO-DEMOGRAPHIC RISK FACTORS FOR MALARIA IN A NEWLY ESTABLISHED SURVEILLANCE REGION IN THE CHITTAGONG HILLS TRACTS OF BANGLADESH

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Until recently, the Chittagong Hills have been hyperendemic for malaria but in recent years has been hypoendemic. A new study initiated in two

unions (population=20,563) near Bandarban, Bangladesh in 2009 was designed to improve knowledge of malaria transmission, to monitor malaria interventions, and serve as an area for developing new control strategies. The project included: (a) demographic surveillance system, (b) periodic surveys of knowledge, attitude, and practice, (c) geographic information system, (d) weekly active and continuous passive surveillance for malaria infections, (e) monthly mosquito surveillance, (f) daily weather measures. The program included both standard and molecular methods for case detection. Between October 2009 and January 2011, 151 cases of malaria were detected. 83% were symptomatic infections (97% chloroquine resistant *Plasmodium falciparum*, 2.5% *P. vivax,* and 0.8% mixed infection). Malaria infections were highly clustered geographically and seasonally. Risk factors associated with higher malaria rates included age, pregnancy, education and occupation. In univariate analysis, there was increased odds of high-season malaria disease in children aged 1-4 (95%CI 1.1, 3.8) and 5-14 (95%CI 1.7, 4.0) compared with those aged >14. Tribal people had a 4-fold higher odds compared to Bengalis (p=0.019) living in the same unions. These effects were independent and of similar magnitude and significance in a multivariate model. After controlling for the effects of union of residence, age and sex, there was an increased odds of malaria amongst people doing day labor (2.5-fold, p=0.046) and a hillside agriculture practice by tribal groups called "jhum" (4.3-fold, p=0.002), but not other agricultural occupations (p=0.304). These results reveal socio-demographic risk factors for symptomatic malaria during the high-season in this Chittagong Hills surveillance region that will: (1) serve as a basis for future hypothesis-driven epidemiological studies, or (2) target future intervention strategies to high risk groups.

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INCIDENCE OF MALARIA IN THE FIRST YEAR OF LIFE IN A HIGH MALARIA TRANSMISSION AREA IN GHANA

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Malaria in pregnancy is a risk for abortions and low birth weight. Placental malaria may also have an impact on the infants susceptibility to parasitemia, clinical malaria and anemia. A prospective birth cohort study was carried out among pregnant women and their infants to determine the incidence of malaria and its effects on the infant's health in a high malaria transmission area in Ghana. At birth, placental biopsy was taken to determine placental malaria. The infants were followed up for a period of one year. Each infant was followed up monthly for malaria parasitemia, anemia and passively for clinical malaria. The mean total IgG levels to the *Plasmodium falciparum* CSP antigen (NANP6) were also determined by ELISA in a subset of the birth cohort at birth, 3 and 6 months of age. A total of 2810 pregnant women were identified and followed up till birth. The average age of mothers was 27 years with 25% and 75% being primigravidae and multigravidae, respectively. Twenty-two (22) percent of the pregnant women were in the very poor quintile of socioeconomic status. The coverage of intermittent preventive treatment with at least one dose of SP was 95% and insecticide treated net (ITN) use was 38%. The prevalence of placental malaria was 37.3%, 95% CI 35.22-39.44. A total of 1605 infants contributed to 1079.0 PYs of followed up. The mean coverage of ITN use among the infant cohort at any point of contact was 36%. The incidence of malaria parasitemia was 0.50PYRS (95% CI 0.46 -0.55 unadjusted), 0.68PYRS.(95% CI 0.47-0.97 adjusted); incidence of anemia was 3.34 95% CI 3.17-3.52 unadjusted, 4.13 95% CI 2.22 -7.68 adjusted and incidence of clinical malaria was 0.22 95% CI 0.20 - 0.25 unajusted, 0.45 95% CI 0.16 -1.23 adjusted. The mean total IgG levels to CSP at birth, 3 mo and 6 mo were 1091.20 95% CI 952.21 -1249.51; 203.9 95% CI 174.11 -238.89, 179.4 95% CI 147.53 -218.18 respectively and the mean total IgG levels to GLURP RO at birth, 3 mo and 6 mo were 257 95% CI 187.38, 352.46, 38.5 95% CI 28.38, 52.21, 36.9 95% CI 26.56, 51.20 respectively. In conclusion, the incidence of malaria among infants in the first year of life is low while the incidence of anemia is high. In addition IgG levels at birth was higher to CSP than GLURP and decayed to lower levels at month 3 and month 6th in the infant cohort.

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ASSESSING THE CLINICAL EFFICACY OF MALARIA VACCINES IN TRIALS AND THEIR IMPACT IN THE FIELD: A MULTIFACETED APPROACH TO USING SIMULATION MODELS FOR PREDICTION

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Assessing vaccines against *Plasmodium falciparum* in clinical trials and predicting their impact after licensure comes with many complications and open questions. RTS,S, the vaccine furthest in clinical development with initial Phase III trial results expected before the end of the year, may be licensed in the next few years. During trials of this type of vaccine and others (such as vaccines directed towards the blood-stage cycle of malaria) important questions are raised concerning how to adequately define endpoints to assess efficacy, and if the impact of the vaccine will vary with transmission setting, age-group immunized, or delivery strategy. In this work, we examine these questions using an ensemble of simulation models for malaria epidemiology and control. We discuss the advantage of model ensembles to quantify uncertainty about predictions and show how uncertainty in predictions varies with transmission setting, with simulations suggesting greater confidence in predictions of health effect for lower transmission settings than for higher ones. We discuss simulation results that show the choice of clinical endpoints used to assess the efficacy of vaccines in trials, especially for blood-stage vaccines, impacts the perception of the success of a vaccine. In addition, we show how ensemble models might be used to study new approaches for delivery of pre-erythrocytic vaccines like RTS,S, with results indicating mass vaccination strategies, even at modest coverage, substantially reduce transmission compared to immunization of infants alone and contribute to much greater health effects per dose. Our multifaceted approach to modelling and simulation of malaria vaccines, with multiple models for predictions at the population level and within-host level, offers not only a pragmatic way to predict their impact and cost-effectiveness, but also allows decision makers to appraise alternatives for delivery and for efficacy assessment to those considered in trials.

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MICRO-GEOGRAPHIC ENVIRONMENTAL RISK FACTORS FOR CHILDHOOD MALARIA DURING THE DRY SEASON IN LIWONDE, MALAWI, 2010

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Despite the importance of environmental factors to *Plasmodium* transmission and the re-emergence of spatial epidemiologic methods for studying malaria, the role of fine-scale environmental heterogeneity in rural locations has been relatively unexplored, particularly during periods of seasonally low transmission. During the dry season of 2010 in Liwonde, Malawi, children attending Machinga District Hospital's (MDH) under-5s clinic were studied for household environmental characteristics that predicted malaria. *P. falciparum* infection in children were determined using Parachek® Pf Rapid Diagnostic Test (RDT), with demographic and environmental data collection occurring at the residence. House location

and elevation were recorded with a global positioning system (Garmin eTrex Venture® HC), as were locations of water sources. Four distinct land cover categories, along with materials used in house construction, were assessed by direct observation. In multivariate logistic regression, children who lived within a 25 m radius of actively cultivated agricultural land were more likely to have malaria when compared to children who did not, after controlling for age and elevation (odds ratio = 2.39, 95% confidence interval: 1.12, 5.10). No spatial clustering or autocorrelation of malaria cases was found, perhaps indicating that increased risk from proximity to agriculture is independent of geographic location across the study region (Moran's I = -0.20, P value = 0.66). This study provides preliminary evidence of continued *Plasmodium* transmission during the dry season, and of various environmental factors that influence malaria risk in rural Malawi. The implications for attempting to eliminate malaria are explored.

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STANDARDIZING A NATIONAL MALARIA BULLETIN FOR TAPPING THE POTENTIAL OF ROUTINE HEALTH MANAGEMENT INFORMATION SYSTEM DATA IN AFRICA: PROCESS AND RESULTS FROM ZAMBIA

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For high-burden African countries, routine information systems offer many often untapped opportunities to present local-level information on malaria impact, logistics and service delivery. The information needs and opportunities of the Zambia National Malaria Control Programme are reviewed with respect to the routinely-reported health information system. A standard indicator set, methods of data collection, data systems for storage and most importantly, analysis and presentation of results were developed from national HMIS system data to support the information needs of an ever-complex malaria epidemiological situation over the period 2001-2010. The resulting national malaria bulletin serves, based on a consolidated District Health Information System 2.0 information system platform, as a model for malaria endemic-African countries to improve analytic capacity of the Ministry of Health and malaria control partners to promote best-practice malaria monitoring, evaluation, and surveillance for improved decision making for malaria control at national and local levels.

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USING CENSUS METHODS TO PREPARE FOR EVALUATING THE IMPACT OF UNIVERSAL SCALE-UP OF MALARIA INTERVENTIONS: CASE OF LIKOMA DISTRICT, MALAWI

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Malaria Indicator Surveys (MIS) and Demographic Health Surveys (DHS) are traditionally used to document coverage and impact of interventions on disease burden. Malawi carried out its first MIS in 2010, but sampling excluded Likoma Island, which is situated in Lake Malawi. The Malawi National Malaria Control Programme planned to pilot a campaign to achieve universal coverage of long-lasting insecticide treated nets (LLINs) in 2010 in Likoma district; comprising Likoma and Chizumula Islands. We conducted a population census during the low transmission season prior to distribution and tested all children under five for malaria parasites and severe anemia. A repeat of the enumeration and testing is planned after the campaign. The census was carried out using a shortened version of the MIS questionnaire programmed on personal digital assistants. We geo-referenced all households to analyze spatial patterns. We enumerated 2,189 households, which included a total population of 11,079 including 1330 under five years children. Insecticide-treated-net (ITN) coverage was higher on Likoma Island (64.9%) than the national estimate from the 2010 MIS (58.2%). ITN use among under five years old children, was lower (45.9%) than the 2010 MIS estimate (55.4%). ITN use among pregnant women was 35% compared to 49.4% in the 2010 MIS. Bio-marker samples were collected from 904 out of 1330 children (68.0%) aged 6 to 59 months, of which 835 (62.8%) were included in parasitological reading. Samples were not collected from over 30% of children due to frequent travel to the mainland. Malaria prevalence was 9.2% and severe anaemia (Hb < 8g/dl) prevalence was 7.5%. Spatial analyses are currently being conducted to assess disease clustering. As National Malaria Control Programmes is planning universal coverage of LLINs and other interventions, it is important to explore new and creative ways to best measure the impact of interventions. In locations with small confined populations, a population census that includes malaria parasite testing of all children under five provides important data on the spatial distribution of disease. We conducted this census in the dry season, when malaria parasite infections are more likely to be spatially clustered. When combined with health facility incidence data, census data such as ours can help elucidate disease dynamics and support operational research into how best to improve control.

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MALARIA CONTROL INTERVENTIONS: A COST-EFFECTIVENESS ANALYSIS

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With a human burden of more than 500 million people every year, the need for cost-effective malaria control is outstanding. The objective of malaria control is to significantly reduce the rate and number of cases of parasitic infections and clinical malaria. This study analyzes a number of control interventions and presents a template for decision makers looking to reduce the burden of malaria in a cost-effective manner.Twenty two malaria control interventions are analyzed. Cost data from WHO-CHOICE were used to calculate the current DALY value was calculated using twelve scenarios, differing in β (age weighting parameter), K (age weighting modulation factor) and r (rate of discounting). Standard life expectancy was calculated using WHO tables. Total current DALYs were calculated using the current DALY value and age group population data for each country from the United Census Bureau International Data Base. DALYs averted were then calculated by multiplying the coverage rate of an intervention and its efficacy with the total current DALY. Efficacy rates were determined using WHO cited literature but were varied to model three scenarios for each intervention. The cost-effectiveness of interventions was analyzed by considering the average cost per DALY averted. The most cost-effective intervention under every scenario is case management with artemisinin-based combination therapy; the least cost-effective intervention is insecticide-treated bed nets. Results from the cost-effectiveness analysis suggest that marked increases in funding for and supplies of insecticide treated bed nets may be misguided from a cost-effectiveness standpoint. Recent pushes to scale up artemisinin-based combination therapy policies fall in line with the findings of this study, especially in sub-Saharan Africa.

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UNEXPECTED PATTERNS OF PRIMARY SYMPTOMATIC MALARIA AND NON-MALARIA ILLNESS EMERGE IN A BIRTH COHORT OF GHANAIAN CHILDREN DESPITE WIDESPREAD USE OF INSECTICIDE-TREATED BEDNETS (ITNS) AND STANDARD ARTESUNATE COMBINATION THERAPY (ACT)

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To gauge the extent and impact of a national malaria prevention/control strategy based on insecticide-treated bednets (ITNs) and Artesunate Combination Therapy (ACT), all-cause illness, symptomatic malaria, and death was monitored in a cohort of 2,279 live births from March, 2006 to October, 2008, in the Kassena-Nankana District of northern Ghana. Ownership of ITNs in this cohort rose from 76% at 4 mos. to 95% at 16 mos. and malaria prevalence in young infants was 15% in households with an ITN compared to 31% in households without (OR: 2.7; 95% CI: 2.0, 3.6). Despite high ITN coverage and free, standard Artesunate + Amodiaquinetreatment for uncomplicated malaria, *Plasmodium falciparum* was detected in 44% (608/1,375) of all inpatient (IP) and 35% (6,327/18,223) of all outpatient (OP) visits. Several unexpected findings emerged: 1) Firstborns had a significantly lower incidence of primary symptomatic malaria and a longer time to this event than infants of multigravid mothers, but suffered a higher incidence of non-malaria illness; 2) Between the two dominant ethno-linguistic groups (Kassem and Nankam), onset of primary malaria illness in infants of Nankam ethnicity came two months earlier and infections amounted to an estimated 500 more OP malaria cases/thousand, but paradoxically, these children accrued grossly lower annualized rates of severe malaria and non-malaria illness requiring hospitalization; 3) More high parasitemias were seen in females, but males accounted for a significantly greater proportion of severe malaria anemia cases (2.6% vs. 1.9%; P = 0.04). Malaria was associated with 36% of cohort deaths that occurred in the hospital but case fatality rate for children with malaria was 2.5% compared to 5.7% (P = 0.02) for admissions with no detectable parasitemia. All symptomatic malaria was seen in the post-neonatal period, associated with 20% of IP deaths in infants, 53% of IP deaths in children >12 mos., and collectively in 36% of all IP deaths. Our cohort all-cause infant mortality rate of 37/thousand, which may owe ~20% of its deaths to malaria, is well below the infant mortality rate of 68/thousand for the Upper East Region, and the national rate of 71/thousand calculated by the Ghana Multiple Indicator Survey in 2006. These results, derived from real-life practices and outcomes in a rural community may be indicative of new trends in malaria prevention and child survival that are occurring widely in sub-Saharan Africa.

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ASSOCIATION BETWEEN VECTOR CONTROL COVERAGE, CLIMATE VARIABILITY AND THE SPATIAL DISTRIBUTION OF MALARIA AT THREE TIME POINTS IN ZAMBIA

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Three malaria indicator surveys (MIS) have been conducted in Zambia since 2006 to evaluate intervention scale-up. Coverage of insecticide-treated mosquito nets (ITNs) and indoor residual spraying (IRS) has increased since 2006. However, while malaria infection and anemia prevalence in children younger than 5 years dropped in 2008, results from 2010 indicate that levels have rebounded in several parts of the country. We sought to ascertain the relative effects of ITN coverage, IRS, and climate variability on the spatial distribution of malaria infection and anemia in 2006, 2008, and 2010. We fit Bayesian geostatistical models to assess the effect of intervention coverage on malaria infection and severe anemia prevalence, while adjusting for climatic and socioeconomic factors. We assessed the spatial dependence of disease distribution through time with spatial random effects for each survey. Model fit was conducted with Markov chain Monte Carlo simulation. Malaria infection and severe anemia prevalence rose from 2008 in six of nine provinces, and from 12% to 20% across rural areas nationally. Parasite prevalence increased by the largest percentage in Luapula (132%), Northern, (97%), and Eastern (137%) provinces. Household ITN possession fell 31% in Luapula province and 29% in Northern province, but remained constant in Eastern province. Parasite prevalence also increased in Central (by 19%), Western (96%), and Copperbelt (22%) provinces, even though ITN coverage also increased, by 45%, 121%, and 9%, respectively. 20-day cumulative rainfall estimates two months before each survey were positively associated with odds of malaria parasite infection; rainfall was highest preceding the 2010 survey, and lowest in 2008. In 2010, greater ITN age was associated with greater odds of malaria parasite infection. Spatial dependence increased with each survey year. These results suggest that a combination of climatic factors, lower ITN coverage, and ITN age contributed to the rebound in parasite infection prevalence in some areas. Unusual rainfall patterns in the early part of 2010, perhaps related to moderate El Niño conditions, may have contributed to this increase. We emphasize the importance of accounting for climate variability and spatial heterogeneity when using cross-sectional data for malaria evaluation efforts.

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FACTORS AFFECTING ACCESS TO ACT TREATMENT FOR UNCOMPLICATED MALARIA IN WESTERN KENYA

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Effective case management is central to reducing malaria mortality and morbidity worldwide, but only a minority of those affected by malaria, have access to prompt effective treatment. In Kenya, the treatment policy for malaria has changed from chloroquine (CQ) to sulphadoxine-pyrimethamine (SP) as the first-line antimalarial drug for uncomplicated malaria in 1998, and then from SP to artemisinin-based combination therapies (ACTs) in 2004. Despite this, these three classes of antimalarial drugs are being used by residents in endemic areas in Kenya; however the extent of ACT usage is unknown. We have surveyed 1,100 households of 5,775 individuals and 1,930 antimalarial prescriptions/treatments in three district in western Kenya in 2003 and 2010. We found that the SP and amodiaquine (AQ) based antimalarials accounted for 88% of prescriptions/treatments in 2003, 4% of the cases were treated with quinine (QN) and the rest were with CQ. Malaria treatment-seeking occurs mostly in the formal sector, i.e., government-run health centers and hospitals. In 2010, 58% of the cases were treated in government-run health centers/hospitals. Overall, only 60% of the antimalarials used in 2010 were first-line government recommended drugs. Shortage of ACTs in stock at government hospitals and clinics and less cost for CQ and SP drugs in private sectors are the major reasons for the patients to obtain them from the private sectors.

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HOUSEHOLD SURVEYS OF MALARIA INFECTION, FEVER AND ANEMIA IN A BIRTH COHORT OF GHANAIAN INFANTS REVEAL TRENDS IN ITN OWNERSHIP, BENEFIT AND PARENTAL ACTION IN SEEKING HEALTH CARE FOR THE BABY

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Prospective study of illness and death in a birth cohort of 2279 Ghanaian infants was complemented by cross-sectional surveys timed to correspond with the end of low (April-May) and high (Nov.-Dec.) malaria transmission seasons. Home visits during Oct.-Nov. 2006, when infant's age averaged 4 mos., found ITN ownership in 75% of households (range: 62-83%). Malaria prevalence associated with ITN ownership was 15%, ranging from 3% (town) to 21% (non-irrigated, rural). Malaria prevalence among infants lacking ITNs averaged 31% and ranged from 12.5% (town) to 42% (irrigated rural). Although ITN ownership was similar in the four ethnic groups (74-89%), malaria prevalence was significantly higher among Nankam infants (22%; P = 0.011). Fever was reported by mothers in 29% of *Plasmodium falciparum* infections but actually measured in 13.5% of these cases. Prevalence of shaking, diarrhea, vomiting, cough, or breathing difficulty was similar in infants with and without parasitemia but fever and anemia was more prevalent in children with *P. falciparum*. Records showed that only 27% of infants with parasitemia were brought to clinic for treatment within 7 days of the home visit. Severe anemia, fever and older age distinguished those brought to clinic. Analysis also revealed that malaria in these infants was associated with multiple maternal factors: older age, less education, no education, fewer ANC visits, and no use of ITN during pregnancy. By Oct.-Nov. 2007, when children averaged 15 months old, ITN ownership had increased to 95% but prevalence of *P. falciparum* infection was nearly twice greater than in the previous year, gametocyte carriage rate was more than doubled (9.6% vs. 3.7%; P < 0.001), high parasitemias >20,000/µL were four times more prevalent (13% vs. 3%; P < 0.0001), and anemia (Hb <8.0) had increased in both parasitemic (45.5% vs 10.3%; P < 0.0001) and parasite-free (15% vs. 5.2%; P < 0.0001) children. Records showed that 38% of children with parasitemia were brought to clinic for treatment within seven days of the home visit. Among 259 children with malaria who did not report there were 64 parasitemias >10,000/µL, 27 with fever, and 6 with severe anemia. It appears that the benefit of ITNs was offset by heightened immunological susceptibility of these young children.

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PERFORMANCE OF A RAPID DIAGNOSTIC CARD TEST FOR DETECTION OF SINGLE- OR MULTI-SPECIES *PLASMODIUM* INFECTIONS AMONG RESIDENTS OF SOUTHERN COAST PROVINCE, KENYA

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As part of a study of polyparasitism, we examined the utility of rapid diagnostic testing for classification of malaria infection status. Diagnostic cards are readily deployable but may fail to accurately identify all prevalent cases and may miss non-*falciparum* or multi-species *Plasmodium* infections. In our study, adult and pediatric samples were collected in two villages in Msambweni District, Kenya, and tested for malaria both by ICT card and multiplex PCR-Ligase Detection Reaction (LDR). LDR is an accurate and sensitive method to detect malaria that can discriminate among single or concurrent *P. falciparum*, *P. malariae*, *P. vivax* and *P. ovale* infection. By LDR, 38% of Milalani samples (267/704) and 19% of Nganja samples (92/481) were positive for at least one *Plasmodium* species. Of these positives, 44% and 48% were positive for *P. falciparum* alone in Milalani and Nganja respectively, 20% and 17% positive for *P. malariae* alone, 7% and 2% positive for *P. vivax*, and 7% and 4% for *P. ovale* alone. Pediatric cases were more common in Milalani (32%) than Nganja (23%, p=0.014), however Milalani sampling occurred in July 2009 (early dry season) and Nganja collection in April 2009 (mid wet season). The difference between villages in adult cases was not significant. As a screening tool, ICT cards (designed for detection of *P. falciparum*) had sensitivity of 43% for *falciparum*, 10% for *vivax* and 0% for ovale and malariae. Specificity was 99% for all four species. Specificity remained the same for *falciparum* in single *vs.* multi-species infections (99%), but sensitivity lowered to 29% when non-*falciparum* species were present. ICT positive predictive value (PPV) for *P. falciparum* was 86% and negative predictive value (NPV) was 90%. For single-species *P. malariae* infection, the PPV was 0% and NPV was 92%. PPV for isolated *P. vivax* was 15% and NPV was 98%, and PPV for *P. ovale* was 0% and NPV was 97%. We conclude that accurate attribution of infection-associated morbidities will require the more sensitive and comprehensive PCR approach to molecular detection of infection.

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INTERVENTION IS NOT ENOUGH: FUNCTIONAL SOCIAL NETWORKS ARE CRITICAL TO THE SUCCESS OF COMMUNITY LEVEL MALARIA CONTROL

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Despite recent trends showing a decrease in the malaria burden in Senegal, there is still substantial regional variation. In the Tambacounda region malaria accounts for 13.5% of all outpatient consultations and 22.4% of mortality; parasite prevalence in children under five is 23.4% compared to the national figure of 5.7%. Coverage with key interventions, including access to prompt and effective treatment with artemisinin combination therapy (ACT), is low. The population of Tambacounda is widely dispersed and access to health care is limited. The health system in Senegal provides community outreach services at the village level through health huts staffed by community health workers (CHW) and managed by village health committees (VHC). However, in Tambacounda most community level services are no longer functional. The Mobilise Against Malaria programme rehabilitated 24 non-functional health huts and implemented an intervention to improve access to prompt and effective malaria treatment in these health huts. Midterm household survey data found limited overall improvement in prompt and effective treatment of febrile children under five. Contextual data suggest several reasons for these results but monitoring data indicate that some health huts perform better than others despite sharing many contextual factors. We conducted case studies of 3 well performing and 3 poorly performing health huts to investigate the facilitating factors and barriers to their effective operation. Health huts were identified using a combination of objective and subjective functionality criteria (including treatment indicators, utilisation rates, ACT stock, completeness of reporting). In-depth interviews were conducted with the CHW and VHC of the 6 health huts as well as their district supervisors. The health hut system is centred on the CHW; however the CHWs are themselves at the centre of a complex network of social relationships that function to influence the success of the health hut as a community-level service provider. These critical relationships and the broader implications of the findings are discussed.

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MALARIA INFECTION IN KENYAN PREGNANT WOMEN IS ASSOCIATED WITH LOW FETAL AND NEONATAL BIRTH WEIGHTS

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Low birth weight is associated with malaria exposure in pregnancy; however, little is known about fetal growth *in utero* among malaria-exposed fetuses, which has implications for outcomes. We sought to evaluate fetal growth, birth weight and anthropometrics associated with malaria exposure among infants born to Kenyan pregnant women. From 2005 to 2007, pregnant women in Kenya were recruited at antenatal care (ANC) and those with term live births delivering at the study hospital who consented enrolled. Women received IPTp and were tested for malaria by microscopy at presentation and delivery. At least 1 fetal ultrasound (US) exam which measured fetal head circumference (HC), biparietal diameter, abdominal circumference, and femur length to generate fetal weight was performed. Fetal growth measures stratified by gestational age at first US were compared using t-tests between fetus/infants with or without evidence of maternal malaria infection at first ANC. At birth, neonatal weight, length and HC were obtained and tested between those with and without malaria infection at first ANC. 485 women were enrolled and 29 were malaria-positive at first ANC. Fetal weights were lower in fetuses of mothers with malaria infection in early pregnancy (<22 wks) compared to <22 wks fetuses of mothers without malaria infections (324 vs 395 g, respectively, p=0.04,). At > 23wks, while the estimated fetal weights were lower for malaria-exposed, no statistically significant differences were found. Birth weight of neonates born to mothers with vs. without evidence of malaria infection at first ANC was significantly lower, 2836 g (SD 397) vs 3019 g (SD 432), respectively, p=0.03. Neonatal HC and length were not significantly different between malaria exposed and malaria not exposed neonates. Our results suggest that infants exposed to malaria *in utero* had lower fetal and birth weights compared to infants born to mothers with no evidence of malaria infection. Given the small sample size, further research is needed.

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MALARIA AND ANEMIA PREVALENCE AND LONG LASTING INSECTICIDAL NET (LLIN) OWNERSHIP AND USE MEASURED IN REPRESENTATIVE HOUSEHOLD SURVEYS IN PLATEAU AND ABIA STATES, NIGERIA IN 2010

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There have been few recent surveys of malaria prevalence and net coverage in Nigeria. In September 2010, The Carter Center worked with the ministries of health of Abia (south east Nigeria) and Plateau (north central Nigeria) states to conduct a modified Malaria Indicator Survey. The survey was completed prior to mass LLIN distribution campaigns in these states. In 60 systematically selected clusters (census enumeration areas or segments thereof) of 25 households each per state, the average household size was 4.4 persons in Abia (1429 households, 5764 persons) and 6.2 in Plateau (1379 households, 8331 persons). Children <10 years of age in all households were tested for malaria and anemia, and all persons in every third household were tested for malaria. The percentage of households owning at least one net was much lower in Abia (7.2%) than Plateau (35.1%). The majority of nets observed were LLIN: 90.2% (N=147) in Abia and 93.2% (N=811) in Plateau. The percentage of persons using nets the previous night were: Abia: 2.9% of all ages, 5.5% of children under 5 years and 4.7% of pregnant women; Plateau: 15.5% of all ages, 20.7% of children under 5 years, and 24.7% of pregnant women. Despite lower net use, the overall crude malaria prevalence (by CareStart PAN/Pf RDT) was lower in Abia (36.2%, 95% CI 34.3-38.0%, N=2614) than in Plateau (45.2%, 95% CI 43.8-46.8%, N=4212). Age-adjusted prevalence was 29.7% in Abia and 36.9% in Plateau. Age specific prevalence peaked in the 5 to 9 year age group at 52% (95%CI 48.0-55.9%) in Abia and 61% (95% CI 58.3-63.8%) in Plateau, with second highest prevalence in the 10-14 year age group (Abia 48.9%,95% CI 42.2-55.6% ; Plateau 54.9%, 95% CI 50.0-59.8).The percentage of children <5 with anemia (hemoglobin < 8 g/dl) was higher in Abia (20.5%, 95% CI 17.7-23.5%, N=785) than Plateau (9.9%, 95% CI 8.3-11.6%, N=1367). The results indicate that malaria is highly prevalent in these two states, and that LLIN ownership is low. The national campaign now underway to provide 2 LLIN to every household in Nigeria is a welcome development.

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NOVEL STRATEGIES LEAD TO NEAR ELIMINATION OF MALARIA IN PREVIOUSLY HIGH-RISK AREAS IN SURINAME, SOUTH AMERICA

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Suriname was a high malaria risk country before the introduction of a new 5-year malaria control program in 2005, the Medical Mission Malaria Program (MM-MP). Malaria was endemic in the forested interior, where the stabile village communities were most affected. The interventions of the MM-MP included new strategies for prevention, case management, behavioral change communication (BCC)/ information, education and communication (IEC), and strengthening of the health system (surveillance, monitoring and evaluation and epidemic detection system). The interventions of the MM-MP are reviewed and related to the Performance Indicators established by the donor. The changes in the national malaria situation during the years of the MM-MP, based on analysis of the national databases, are discussed. After a slow first year with non-satisfying scores for the performance indicators, the MM-MP truly engaged in its intervention activities in 2006 and kept its performance up until the end of 2009. A total of 69.994 long-lasting insecticide treated nets were distributed and more than 15.000 nets re-/impregnated. Residual spraying was performed in high risk areas and over 10.000 people were screened with Active Case Detection in outbreak or high risk areas. Additional notification points were established and the national health system was strengthened. Malaria vector populations, monitored in sentinel sites, collapsed after 2006 and the number of national malaria cases decreased from 8618 in 2005 to 1509 in 2009. Malaria transmission risk has shifted from the stabile village communities to the mobile gold mining communities, especially those along the French Guiana border. The novel strategies for malaria control introduced in Suriname have led to a significant decrease in the national malaria burden. The challenge is to further reduce malaria using the available strategies as appropriate in the affected areas and populations. Elimination of malaria in the country will require a thorough understanding of transmission dynamics and a dedicated investment in key effective interventions.

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MALARIA FORECASTING: PAST WORK AND FUTURE DIRECTIONS

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Since 1911, when Christophers quantified the strong correlation between malaria incidence and rainfall, researchers have sought to discover other sources of spatial and temporal variability of malaria. The field of malaria incidence forecasting has incorporated predictors responsible for this variability although the approaches are quite diverse. We conducted a scoping review to summarize the heterogeneous field of malaria forecasting and describe the modeling approaches and methods of model evaluation. Two reviewers identified articles by using medical subject headings and key terms to search electronic databases and grey literature, including articles that presented models predicting human *Plasmodium falciparum* malaria incidence or prevalence. The initial search captured 213 different citations, 46 (22%) of which were reviewed. Most models predicted malaria incidence in Africa (72%), with 10 studies (23%) conducted in Kenya. Models mostly included two predictors (52%), which were the previous month's cases of malaria and rainfall. Typically, researchers used ARIMA-based models, which performed optimally within the first 3 months and evaluated using magnitude of correlation between predicted and observed incidence or prevalence. Nearly all malaria prediction models have narrowly focused on a small number of environmental predictors despite the importance of other malaria risk factors (land use, bednets, indoor residual spraying, drug resistance). To advance malaria prediction forecasting, we need more comprehensive models as coarse models will not provide the precision necessary to guide targeted intervention efforts.

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MONITORING MALARIA IMPLEMENTATION COST - THE CASE OF ARTEMETHER-LUMEFANTRINE

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Tanzania has adopted Artemether-Lumefantrine (Alu) as the first line treatment for malaria. INESS has introduced a series of studies in Rufiji and Kilombero/Ulanga Health and Demographic Surveillance System (HDSS) sites to evaluate safety and effectiveness of this drug and their related factors. The collection of treatment related costs has provided an opportunity to analyse the household level costs for getting malaria treatment. Here we report findings of assessment of treatment costs in Rufiji HDSS. Data were collected using household survey conducted in Rufiji DSS site. Members with recent fever from the sampled households were asked questions about professions, economic activities, treatment seeking, costs involved and day lost due to illness. 29.6% of patients interviewed reported to be actively engaged in income generating activities. Farming is the main economic activity in the area accounting for 76.3%. A daily median income in the study area was USD.1.4. However, 22.7% of patients and 59% of accompanying persons reported to have lost their income due to illness or escorting patients respectively. The number of days lost due to a single malaria episode ranged 1-14 days for patients. The direct medical costs (drugs, laboratory tests and consultation) were paid by 81% of patients with median payment of USD0.8. Equally, 11.5% of patients paid non medical cost (including food, lodging, telephone and gifts) with median payment of USD0.4. Only 10.4% of patients reported paying transport cost to get to health providers with median payment of USD1.0. A single malaria episode was observed to cost households more than their income. Alternative payment mechanism in the form of insurance should be considered. Strengthening and expanding coverage of community funds in rural areas could offer protection to rural households from paying more than they earn and when they are not able to work.

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FORECASTING AND MONITORING MALARIA RISK IN THE AMHARA REGION, ETHIOPIA

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Malaria is a major health problem in most sub-Saharan Africa countries, including Ethiopia. Mosquito populations and malaria risk are affected by environmental triggers, including rainfall, temperature, and humidity. The main objectives of this study are to compare alternative statistical forecasting models and quantify the lead time of satellite derived environmental variables with malaria outbreaks. Daily rainfall data were acquired from the Tropical Rainfall Measuring Mission (TRMM) with 0.25 degree spatial resolution. Land surface temperature (LST), normalized difference vegetation index (NDVI), and enhanced vegetation index (EVI) were derived from MODIS 8 day and 16 day composites with a 1 km spatial resolution. Actual evapotranspiration (ETa) was estimated by using the simplified surface energy balance method. Monthly malaria case data for the Amhara region were acquired from the Anti Malaria Association (AMA), Ethiopia. Satellite derived indices were aggregated at a monthly resolution to match malaria cases. Using the historical satellite and case data, we explored a variety of time series modeling approaches with combinations of the different variables. We used environmental variables with lags ranging from 1 to 6 months and examined the temporal cross correlations between outpatient malaria cases and environmental variables. The results showed that there were significant correlations based on a two standard error limit with rainfall at a lag of 3 months; nighttime LST and ETa at 1 month; and NDVI and EVI at 2 months. We found that rainfall and nighttime LST were the strongest predictors for malaria risk in the Amhara region of Ethiopia. Forecasts were validated using data withheld from the model fitting. The results showed that the models provide indications of future outbreaks with a lead time of 1 to 3 months. The findings can be used to enhance future operational Malaria Early Warning Systems in the Amhara region, Ethiopia.

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THE DYNAMICS OF NATURAL *PLASMODIUM FALCIPARUM* INFECTIONS

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Natural immunity to *Plasmodium falciparum* malaria has been widely studied, but its effects on parasite dynamics are poorly understood. Acquisition and clearance rates of untreated infections are key elements of the dynamics of malaria, but estimates of these quantities for endemic areas is challenging because of frequent super-infection and imperfect detectibility of parasites. Consequently, information on the effects of host immune status or age on these parameters is fragmentary. An age-stratified cohort of 349 individuals from Northern Ghana were sampled six times at 2 month intervals. High-throughput capillary electrophoresis (CE) was used to genotype the msp-2 locus of all detectible *P. falciparum* infections. Force of infection (FOI) and duration were estimated for each age group using an immigration-death model that allows for imperfect detection. Effects of naturally acquired immunity on the FOI and duration should be reflected in age dependence in these indices, but FOI tends to increase with age, and persistence and chronicity of individual parasite clones is characteristic of all age-groups. Duration peaked in 5-9 year old children, (average duration 319 days, 95% confidence interval 318;320).

FOI tended to increase with age. The estimated multiplicity of infection (MOI) was considerably higher than the observed number of clones, especially in older ages. The main age-dependence is on parasite densities, and acquired immunity therefore appears to control transmission mainly by limiting parasite densities in the human circulation.

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MALARIA PARASITE GAMETOCYTEMIA IN A MIXED MALARIA SPECIES HYPOENDEMIC IN PERU: ENVIRONMENTAL FACTORS AND GENETIC MARKERS THAT PREDICT EFFICIENT *PLASMODIUM FALCIPARUM* TRANSMISSION

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Malaria parasites must convert to its sexual stage for transmission from human to mosquito. Factors regulating if asexual stage trophozoites switch to the sexual stage gametocytes are not known, but in *Plasmodium falciparum* (PF) it is clearly not a linear function of within host trophozoite density. The rate of conversion might be attributable to environmental factors and/or a heritable genetic trait. We have a study in Peru where a high proportion of infections have gametocytes despite overall low transmission, enabling us to study the density and the proportion of gametocytes to trophozoites. In active and passive case detection of c.1,900 individuals from 2003-2007, we detected 456 PF and 956 *P. vivax* (PV) infections. This study focus was PF. We surveyed for PV by microscopy to determine mixed species infections. We also considered febrile illness, age (>14.5 considered adult), and hematocrit levels as they relate to gametocytaemia. We genetically characterized PF parasites using 14 microsatellite (MS) markers. The proportion of infections with gametocytaemia ranged from 18% (year-2004) to 38% (2007). In the adults not in children, there was a stepwise increase in proportion with gametocytes each year. Gametocyte densities were higher in mixed species infections. Using the program Structure, we found six families of related parasites based on the MS markers. One of the six clusters included 56% of infections with gametocytemia. Moreover, with principle component analysis we found that PF infections with a ratio of trophozoite to gametocyte density was >25% were caused by a genetically distinct group of PF parasites. Therefore, in addition to environmental factors for conversion there appears a genetic signature for parasites with high gametocyte conversion in our study population. We are determining if there is a genetic association with PF gametocytemia independent of environment and antibody responses. Our work provides insight into the transmission of PF in Peru and suggests that eradication campaigns could create a reservoir of more transmissible PF parasites.

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SOCIAL DETERMINANTS OF ASYMPTOMATIC MALARIA ANTIGENEMIA IN TROPICAL AFRICA

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In the context of intensifying efforts toward global malaria eradication, understanding social patterns of malaria transmission from asymptomatic populations in endemic zones will become increasingly important. Social determinants of health have previously been linked with child and maternal mortality, but not to asymptomatic malaria carriage. We conducted a cross-sectional study of afebrile, healthy children aged 2 months to 14 years attending well-child and/or immunization visits in the North Kivu province in eastern Democratic Republic of Congo. A total of 656 children across three villages were tested for malaria antigenemia by rapid diagnostic test and parents simultaneously completed survey questionnaires related to demographics, socio-economic status, maternal education, as well as bednet use and recent febrile illness. 19% of children were parasitemic (11%, 22% and 23% in Goma, Butembo and Beni, respectively; p=0.009). Increasing levels of maternal education were associated with a lower risk of malaria antigenemia in their children (p=0.001). Children from households with higher numbers of children under 5 years of age were also more likely to be parasitemic (p=0.035). On the other hand, socio-economic index was not statistically associated with malaria antigenemia (p=0.27). In a multivariable model adjusting for age, recent febrile illness, and bednet use, maternal education and number of children under five in the household remained significant predictors of malaria antigenemia. In summary, children of mothers with low education level and living in households with large numbers of young children appear to be at higher risk of asymptomatic malaria carriage. Social determinants of malaria parasitemia may influence transmission patterns and may be useful tools for targeted control efforts.

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LARGE DECLINE IN *PLASMODIUM FALCIPARUM* AND *P. MALARIAE* INFECTION RATES AMONG WOMEN AND THEIR OFFSPRING IN A COMMUNITY ON THE SOUTH KENYAN COAST FROM 1997-2010

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Expanded malaria control initiatives in Kenya include intermittent preventive treatment during pregnancy, insecticide treated bed-net usage, and availability of artemisinin combination therapies for case management of malaria illness has resulted in dramatic decreases in reported hospital admission for severe malaria. The overall malaria infection rates have presumably decreased as well, however no studies have reported longitudinal malaria infection rates in the same population of women and young children, using sensitive and specific molecular diagnostic methods over the past decade of malaria control interventions in Kenya. Randomly selected archived blood samples obtained from four birth cohorts of pregnant women and their children (starting in 1997 [n=54], 2000 [n=174], 2006 [n=444], 2009 [n=76]) enrolled from the Msambweni District hospital located in Coast Province, Kenya were examined by the same PCR- based molecular diagnostic assays for infection with the four human malaria parasites. Maternal *Plasmodium falciparum (Pf)* infections at delivery (1997 and 2000 cohorts where malaria chemoprophylaxis was infrequently used) and at first antenatal clinic visit (2006 and 2009) were 37%, 40%, 15% and 17% respectively. *P. malariae (Pm)* infections in mothers also declined from a peak of 12% in 2000 to 8% in 2009. Peak *Pf* and *Pm* malaria infection rates in children occurred at 30-36 months of age in all cohorts. At this age infections rates with *Pf* declined from 58% in 2000 to 10% in 2006. Similarly *Pm* declined from 9% to 3% over the same period. Peak *P. ovale* infections remained at ~2% and *P. vivax* was not observed in the cohorts. These results show a profound reduction in malaria transmission consistent with an overall reduction in the burden of malaria in the coastal regions of Kenya.

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PLASMODIUM SPECIES UNDETECTED BY *PLASMODIUM FALCIPARUM* SPECIFIC RAPID DIAGNOSTIC TESTS AMONG FEVER PATIENTS IN ZANZIBAR

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Malaria incidence has decreased markedly in Zanzibar, and the island is considering elimination. However, non-*falciparum* malaria may be particularly difficult to eliminate. Importantly, the routine diagnostic standard in Zanzibar has been a rapid diagnostic test (RDT) based on histidine-rich protein-2, which is produced by *Plasmodium falciparum* but not other malaria species. The use of *P. falciparum* specific RDTs therefore may prevent comprehensive malaria case finding in Zanzibar. We report species data on RDT negative, PCR positive malaria cases in Zanzibar during the 2010 rainy season. We collected blood on filter paper from a random sample of febrile patients who tested negative for malaria by RDT in 6 primary health care facilities in North A and Micheweni District, respectively (N = 595). DNA was extracted with Chelex from dried blood spots in pools of 10, and nested PCR targeting the *cytochrome b* gene was performed. Samples from positive pools were reextracted individually. An *AluI* restriction digest and gel electrophoresis were then performed on positive individual samples for species identification. Of the 595 RDT negative samples, 13 (2%) were positive by PCR. Nine out of the 13 positives (69%) were identified as *P. falciparum*, 3/13 (23%) as *P. malariae*, and 1/13 (8%) as *P. vivax.* Three of 4 subjects with non-*falciparum* infections were from Micheweni and the remaining subject was from North A. This study is the first, to our knowledge, to report *P. vivax* in Zanzibar. With improved control of *P. falciparum* infection on the island and with challenges to control of other species, infection with non-*falciparum* species may make up an increasing proportion of malaria cases on Zanzibar. In this context RDTs including both *P. falciparum* specific and pan-species specific antigens should be considered for improved overall malaria case detection and to ensure that elimination efforts are comprehensive.

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RAPID DEVELOPMENT OF *PLASMODIUM FALCIPARUM* STERILE, INFECTION BLOCKING IMMUNITY OVER SUCCESSIVE INFECTIONS IN PERU SELECTING FOR PARASITE GENETIC DIVERSITY

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The malaria paradigm is that resisting symptomatic and high-density *Plasmodium falciparum* (PF) infections requires years of frequent exposure to develop and continued exposure to maintain. In contrast, in a setting of recent and low transmission in Peru, we see a rapid onset of clinical and anti-parasite immunity. Immunity might be related to the PF genetic diversity. We followed 456 individuals who had a PF infection in one year between 2003 and 2007 and were in our active and passive case surveillance in the following year. We genotyped parasites using the Merozoite Surface Protein-1 (MSP-1 B2) and using 14 MS markers scattered across the 14 PF chromosomes. Considering the 175 individuals who had two infections in these two years, the probability of febrile illness was 85% in the first and 60% in the second infection (p=0.012). The probability of not detecting an infection in the second year (despite having at least 6 active detection visits to find even asymptomatic infection) ranged from 21 to 62%, increasing with number of infections prior to these two successive infections. Febrile illness was associated with having a different MSP1-B2 genotype in the second infection (p=0.041). To further consider genetic diversity and immunity, we genetically characterized 303 PF infections using the MS markers. The overall genetic diversity increased over time, with parasites in the later years having markedly different MS haplotypes. We calculated the number of genetic differences between the first and second infection in each individual. MS markers are not considered under immune selection, but in low transmission they would indicate potential allelic differences in antigens in linkage disequilibrium with (nearby) the MS markers. Infections spaced by <18 months (mos) had an average of 5.3 (se: 0.3) differences. Infections spaced by >=18 mos had an average of 4.0 (se: 0.3) differences. The significant association with time separating infections suggested that parasites causing reinfection within 18 mos had to be more genetically different than those occurring after the immune response might no longer exert a selective pressure against a similar parasite reinfecting. Other tests of genetic differences in reinfections versus differences expected by chance indicated selection for parasite diversity. Our results suggest immunity develops and at least some of this immunity is directed to antigens that have different allelic forms in this population.

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INCUBATION PERIOD OF *PLASMODIUM FALCIPARUM* MALARIA IN ADULT TRAVELERS IN THE UNITED KINGDOM

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The incubation period (from infection to onset of symptoms) for *Plasmodium falciparum* malaria has been shown to vary widely from days to months in adults. Estimation of the incubation period distribution informs both diagnosis criteria in non-endemic areas and the design of passive surveillance in areas where local elimination is been achieved. Data on the duration of holiday, date of arrival in the UK and date of onset of symptoms for 413 adults (over 16 years old) with *P. falciparum* malaria reporting to the Infectious Diseases Unit at Northwick Park Hospital, London, UK between April 1991 to May 2006 were analysed. The mean incubation period was estimated using interval censored survival analysis assuming a Gamma distributional form. The role of self-reported previous malaria, antimalarial use, severity of disease, age and ethnic origin (a key determinant of severity in this population) was investigated. 17% of cases had onset of symptoms prior to arriving in the UK, 40% became ill within the first week of arrival and 5% reported first symptoms more than a month after arrival. The mean incubation period was 20 days (95% confidence interval 11-41days). Important determinants of the incubation period ($p<0.05$) were self reporting of previous malaria (mean 17 days for no previous malaria, 23 days with previous malaria) and severity of disease (mean 16 days in patients with severe disease, 21 days in other patients). This analysis has been performed in a group of adults resident in the UK and abroad who had severe enough symptoms to report to hospital, and these estimates may therefore be biased. Severity is associated with a short incubation period in these patients, and therefore incubation periods may be much longer for mild cases and in immune populations. The degree of variability in incubation periods has only rarely been reported and therefore this study gives useful insights for both the UK and international context.

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HIGHER MALARIA PREVALENCE IN CHILDREN FIVE YEARS AND ABOVE IN LAGOS, NIGERIA

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Malaria is almost invariably ranked as the leading cause of morbidity and mortality in Africa. There is growing evidence of a decline in malaria transmission, morbidity and mortality over the last decades especially in Africa. Reports on malaria prevalence in children in Nigeria are divergent in the literatures. This study therefore reports the trend in malaria prevalence in febrile children in Lagos, Nigeria. This study was conducted at the St. Kizito Primary Health Centre, Lekki, and Massey Street Children's Hospital Lagos State, Southwestern Nigeria. This was a cross-sectional study with a total of 1,211 children, aged 0-12years who presented with fever or history of fever in the last 24 hours at the Outpatient's Department of the Clinics. Among the children tested, 658(54.4%) were males and 553(45.6%) were females. Of the total children tested microscopically, 251 (20.7%) were positive for malaria parasites. Children >1-12years in Age Group III had a malaria prevalence of 25.8%, 11.0% in the Age Group I (0-≤1year) (p=0.001), 16.9% in 0-≤5years and 42.1% in >5-12years in Age Group II (p=0.001). The highest mean parasite density (43,097.6 p/µl) was reported in Age Group III (>1-12years). Most of the malaria positive children (33.9%) had parasite density between 1-500p/µl. There was no significant association in monthly malaria prevalence in the studied children. This study reported a decline in malaria prevalence, which may be attributed to large-scale implementation of malaria interventions. There was a shift in malaria prevalence from the well reported prevalence in 0-<5years to >5-12years. The shift in malaria prevalence to >5-12 year olds may reflect successful implementation of malaria control interventions in under-fives, and underscored the need to extend such interventions to older children and indeed implement universal target in malaria control.

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OCCURRENCE AND PATTERN OF IMPORTED MALARIA CASES IN RIO DE JANEIRO, A NON-ENDEMIC STATE IN THE BRAZILIAN EXTRA-AMAZONIAN REGION

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In Brazil, almost all (99.4%) of malaria reported cases are seen in the Amazon region where *P.vivax* accounts for 82.2% of them. The low specificity of initial clinical presentation, overlapped with other acute febrile diseases and the potential risk of severe malaria represents an extra challenge for unaffected regions. The high lethality rates of malaria in the extra-Amazon (70.8 times higher than in the Amazon region) drew the attention of authorities. A retrospective study was carried out from Jan/07 to Apr/11 in a Reference Center that handles 23% of the malaria cases seen in Rio de Janeiro. From the total of 291 admitted malaria suspect patients, 83(28.5%) had the confirmed diagnosis of malaria. The distribution according to *Plasmodium* species was 58 (69.9%) *P. vivax*, 18(21.7%) *P. falciparum*, 3(3.6%) *P. malariae* or *P. ovale* and 4 (4.8%) *P. vivax/P. falciparum* co-infection. Most of suspected cases were primo-infected men over 25 years old and the total lethality rate was 2.4%. Africa or Asia countries were the likely source of infection for 33(37.5%) between 2007-08 and 77(38.1%) between 2009-11 (OR:1.04; IC:0.59-1.82%). This pattern of imported malaria in Rio de Janeiro is different from the one recorded in the Amazon region and may result from the recent Brazilian economic growth that has increased the presence of construction, mining and oil companies in endemic areas as well as the presence of Brazilian enterprises in major international project, especially in Africa. The authors note the recent increase in the absolute number of suspect cases and in the percentage of *P. falciparum* infections, with annual increment ranging from 15-44%, in the this non endemic region. Although the total number is modest, when compared with those recorded in endemic areas in Brazil, two challenges are set for this scenario to avoid increase in morbidity and mortality: the difficulty of rapid and accurate diagnosis; and the training on the management of potentially severe malaria including multidrug resistant *P. falciparum*, which is extremely different from the observed in the Amazon region.

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CHARACTERISTICS OF THE INFECTIONS BY *PLASMODIUM* SPP. DETECTED BY ACTIVE SEARCH OF CASES IN FEBRILE AND NON-FEBRILE INDIVIDUALS OF THREE ENDEMIC COMMUNITIES IN OLANCHO, HONDURAS, SEPTEMBER 2010

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Public health agencies in Honduras implement malaria control activities by responding to reports of active cases. Previous investigations suggest that subclinical cases of malaria exist in communities throughout the country, but these studies were limited in scope. Failure of health authorities to include subclinical cases in planning malaria interdiction efforts minimizes the effectiveness of control programs. In this study, investigators performed malariometeric surveys to estimate the frequency of subclinical cases in populations of three semi-rural communities located in the Department of Olancho, Honduras. Medical teams visited 30 homes in the communities of Sosa Lobo, Chacon and Villa Antonia, Olancho, to perform the survey. The visits took place from August 29 - September 2, 2010. Participants were interviewed and a cursory physical exam was carried out. Blood samples were taken using finger sticks and a rapid diagnostic tests (RDT) was performed during home visits. Additionally, thick film slides were prepared for microscope evacuation and dried blood filter paper collections were used for evaluating malaria parasites for polymorphic molecular markers. Seventy-one individuals participate in the survey with 19 participants experiencing an episode of febrile illness (26.7%) within 30 days period prior to the home visit. Technicians detected one *Plasmodium vivax* (1.4%) positive when evaluating specimens with RDT. Subsequent microscopic evaluation of the thick films resulted in the detection of four additional cases of *P. vivax* (7.0%). Two of the participants that were thick film positive (2.8%) did not have a clinical history suggestive of malarial infection and could contribute to the persistence of transmission in their communities. The molecular analysis of parasite genomic material from the five positive specimens detected one genotype based on DNA fragment size: markers MSP1 5/6 (~380bp), CSP (~600bp) and GAM1 (~500bp). The analyzed samples demonstrated genetic homogeneity and an absence of polyclonal infections.

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ECONOMICAL RABIES POST-EXPOSURE PROPHYLAXIS: A SIMPLIFIED 4-SITE INTRADERMAL 3-VISIT REGIMEN ON DAYS 0, 7 AND 28

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Human encephalitis caused by a dog rabies virus remains 100% fatal, although on rare occasions, patients have recovered from infection with less pathogenic American bat rabies viruses. Post-exposure prophylaxis is often unaffordable or unavailable in Asia and Africa. The intradermal (ID)

route of vaccination has immunological and economical advantages over IM. Two multiple-site ID regimens (8-site and 2-site) requiring <40% of the standard vaccine have been recommended by WHO for 14 years. They are used successfully in a few places in Asia, but rarely in rural areas where 80% of rabies occurs. ID vaccination is practically unknown in Africa. Pharmaceutical and practical difficulties inhibit ID use together with failure to explain and promote the regimens. A new simplified 4-site ID vaccine regimen could overcome many of these problems (Warrell MJ et al. PLoS NTD 2008; 2(4):e224). The regimen employs the same doses of vaccine and the same schedule as the 8-site method, but can be used with rabies vaccines reconstituted to volumes of 1 ml or 0.5 ml per ampoule. The 4-site regimen consists of a whole ampoule of vaccine divided between 4 ID sites, one on each limb on day 0. Two ID injections are given on day 7 and one on day 28. The ID dose is 0.2 ml or 0.1 ml for vaccines of 1 ml or 0.5 ml per ampoule respectively. The 4-site regimen is as immunogenic as the 'gold standard' 5 dose IM course of vaccine. It requires a total of less than 2 ampoules, only 3 clinic visits and does not rely on expert ID injection technique and so is safer in inexperienced hands. It meets all the WHO requirements and is very economical for both the clinic and patients compared with all other regimens (Hampson K et al. PLoS NTD 2011; 5(3):e982 Note extra data in 'Comments'). Rabies vaccines do not contain a preservative and are not licensed as multi-dose ampoules unless authorised by a national regulatory body. If ampoules of vaccine are shared, ID rabies vaccination is given 'on the doctor's responsibility' in most countries. However without any sharing of ampoules, the 4-site regimen is economical even if only one person is treated: using a total of 3 doses with some wastage. The method is applicable globally wherever financial resources or vaccine supplies are limited or if the number of clinic visits is critical.

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DETECTION AND GENOTYPING OF HUMAN ASTROVIRUS IN NEPAL USING REAL-TIME REVERSE TRANSCRIPTASE POLYMERASE CHAIN REACTION

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Human astroviruses (HuAstVs) cause gastrointestinal disease; eight (1-8) classic human astrovirus (AstV-canonical) serotypes are responsible for acute, nonbacterial diarrhea in children. Recently, two new human astrovirus variants (MLB and VA) have been described in humans. We detected HuAstVs by a real-time reverse transcription-polymerase chain reaction (RT-PCR). One step TaqMan multiplex real-time RT-PCR assays were developed to broadly detect HuAstVs. The most conserved sequence between ORF1 coding for the non-structural protein and ORF2 encoding for capsid protein junction was chosen as the assay target. Three sets of primer and probe were designed with specificity for AstV 1-8, AstV-MLB1- 2, and AstV-VA1-3. Primers were optimized with known positive specimens identified by RT-PCR from a previous study using SYBR Green based real-time RT-PCR. TaqMan multiplex RT-PCR assay was applied to screen stool samples with no previously identified enteric pathogens by standard microbiology for enteric bacteria; EIA for rotavirus, adenovirus, astrovirus, giardia and cryptosporidium; or RT-PCR for norovirus and rotavirus. These stool samples were collected from children aged 3 months to 5 years with diarrhea and non-diarrhea controls in a hospital based study in Nepal. All positive samples were tested for HuAstV typing by individual sets of primers and probe. A total of 634 stool samples, 284 cases and 350 controls, were screened. HuAstVs were detected in 9/284 (3.2%) of cases and 9/350 (2.6%) of controls. All 9 samples from cases were identified as AstV-canonical. On further testing of the samples from controls, seven were identified as AstV-canonical, one as AstV-MLB and one as AstV-VA, respectively. Our results indicated that a real-time RT-PCR assays can be used to detect and genotype human astroviruses.

1400

KINETICS OF CHIKUNGUNYA INFECTION DURING AN OUTBREAK IN SOUTHERN THAILAND, 2009

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The Indian Ocean Chikunguna epidemic reemerged in Southern Thailand in September of 2009. We enrolled forty-five adults with laboratory confirmed chikungunya. Serial blood collections and clinical assessments were performed every two-three days through the acute and convalescent phase of the disease until day 30. Patient symptoms were recorded and antibody responses with viral kinetics were evaluated using PCR and serological assays. The patients mean age was 49 years with a male to female ratio of 1:1.4. Thirty-five (77.8 %) patients were rubber planters. All patients experienced joint pain and 42 (93%) of them involved more than one joint. Interphalangeal joints were the most common affected in 41 (91%) patients. The mean duration of severe joint pain was 5.8 days with 11 (25%) experiencing discomfort through the duration of the study. Rash was observed in 37 (82%) patients, a mean 3.5 days after the onset of symptoms. Patients were positive by PCR for a mean of 5.9 days and the peak viremia was at day 5 with 6.24 log PFU/ml. IgM antibodies appeared on day 4 and peaked at day 7. IgG antibodies first appeared at day 5 and rose steadily through day 24. The understanding of chikungunya disease clinical manifestation, antibody responses and viral kinetics are important for diagnosis and treatment of the disease.

1401

PRE-CLINICAL AND CLINICAL DEVELOPMENT OF A VACCINE FOR THE PREVENTION OF HAND FOOT AND MOUTH DISEASE CAUSED BY ENTEROVIRUS 71

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Hand Foot and Mouth Disease (HFMD) is caused by viral pathogens of the enterovirus genus such as enterovirus 71 (EV71) or Coxsackie A16 (CA16). HFMD is generally a self-limiting disease characterised by fever, small blisters in the mouth, and a rash with blisters. However in a small number of cases, HFMD caused by EV71 can lead to viral meningitis, encephalitis, interstitial pneumonitis or poliomyelitis-like paralysis, and may be fatal. The disease can infect any age group, but is rare in children over the age of 10. EV71 and HFMD are endemic in the Asia Pacific region causing millions of cases in recent years. We are developing a well characterized, multiple dose, highly purified, inactivated EV71 vaccine formulated with alum adjuvant. Pre-clinical studies have shown that this vaccine is highly immunogenic and generates strong neutralizing antibody responses in mice, rats and rabbits. We also have demonstrated that these antibodies are capable of cross neutralizing EV71 sub-genogroups currently circulating in Asia. A double blind, placebo controlled Phase 1 clinical study is being conducted in Singapore to assess the safety and immunogenicity of two different dose levels of the inactivated EV71 vaccine in healthy adults. An update of the clinical trial data will be presented.

1402

SENTINEL SURVEILLANCE FOR INFLUENZA IN PHRAMONGKUTKLAO HOSPITAL IN BANGKOK THAILAND

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Phramongkutklao (PMK) Hospital with the Armed Forces Research Institute of Medical Sciences conducted surveillance to identify, characterize and determine the prevalence of influenza and other respiratory pathogens in Bangkok. Patients ≥6 months who meet the criteria of influenza-like illness (fever ≤38ºC and cough or sore throat within 3 days of onset), without tuberculosis and who were not immunocompromised were eligible. History, physical examination, and nasal swabs for rapid kits were performed. Throat swabs were sent for PCR and viral isolation/characterization. 919 subjects were enrolled, 688 (75%) were ≤ 18 years old; 319 (35%) subjects tested positive for influenza by PCR (72% for influenza A). The pandemic strain was the most prevalent (197 cases). The first peak of influenza occurred in Jan/Feb 2010 with >40% of the cases testing positive, the majority were the pandemic strain. The second peak occurred in Aug 2010 with 56% testing positive; the pandemic strain remained dominant, but influenza B accounted for nearly 40% of the cases. There were 106 admissions; 19% were influenza and none had received influenza vaccine. Of 863 subjects reporting vaccination status only 113 had received it within the previous 12 months, and 27 were influenza positive (17 with the pandemic strain). HA gene sequencing on selective samples revealed that the strains in circulation were similar to the 2009 southern hemisphere vaccine strains. In conclusion, influenza is a significant cause of morbidity at PMK Hospital. The population was largely unvaccinated and the majority of influenza cases were caused by the pandemic strain. Vaccination would likely significantly reduce morbidity.

1403

A CHIKUNGUNYA VIRUS HIGH FIDELITY VARIANT LOSES FITNESS IN MOSQUITOES AND MICE

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The error rate of RNA dependent RNA polymerases (RdRp) strongly affects the mutation frequency in a population of viral RNAs. Previously, we used a high fidelity variant of an RNA virus (poliovirus) to illustrate the importance that mutation frequency plays in virus fitness and adaptability *in vivo*. Since arboviruses replicate within very different hosts, the need to generate such genetic diversity may be even more significant than for single ost RNA viruses. Using chikungunya virus (CHIKV), we describe an arbovirus fidelity variant isolated in mutagen treatment with a single C483Y amino acid change in the NSP4 RdRp gene that increases replication fidelity. The increase in fidelity does not have significant costs in terms of replication and RNA synthesis, but shows significant fitness costs *in vivo*. Compared to wild type CHIKV, the higher fidelity population presents reduced infection and dissemination in mosquitoes. Furthermore, viremias in newborn mice are truncated and organ titers are significantly lower. These results indicate that increased replication fidelity and reduced genetic diversity negatively impact arbovirus fitness in invertebrate and vertebrate hosts, a factor that may explain why RNA viruses maintain error-prone RdRp genes.

1404

SMALL MOLECULE INHIBITORS OF VENEZUELAN EQUINE ENCEPHALITIS VIRUS INFECTION IDENTIFIED USING HIGH THROUGHPUT PHENOTYPIC SCREENING OF THE VACCINE STRAIN TC83

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Currently, there is no approved vaccine or therapeutic for prevention or treatment of infection by Venezuelan equine encephalitis virus (VEEV) in humans. Antiviral therapeutics are highly desired for treatment of VEEV infection. We have developed a screening assay in 96- and 1536-well formats to identify antiviral compounds against TC-83, a BSL-2 live attenuated vaccine strain (IND stage) of VEEV. Briefly, primate kidney Vero cells are infected with TC-83 and after 48 hours, cell viability is assessed by measuring cellular ATP levels. The assay was miniaturized to 1536-well plate format and screened against 7,243 unique bioactive small molecules to identify 33 initial actives. The activities of a subset of compounds have been tested in orthogonal *in vitro* assays against VEEV TC-83, VEEV Trinidad (TrD) strains and additionally EEEV and WEEV. We have demonstrated that the approach of using authentic virus, even a surrogate vaccine strain can identify inhibitory compounds against VEEV. Furthermore, the approach can identify molecular targets critical for VEEV infection that could be further developed for human-use antiviral drugs.

1405

BURDEN AND EPIDEMIOLOGY OF ROTAVIRUS DIARRHEA: RESULTS OF A PREVALENCE STUDY IN NIGER

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Diarrhea is still the second leading cause of death in children under 5 years of age in developing countries, representing nearly one in five child deaths. Rotavirus is the most common etiologic agent of severe diarrhea and vaccines are readily available. However, knowledge about the disease burden as well the circulating strains is still lacking in many countries to make informed decisions about vaccine introduction. The regional hospital and ten health centers of Maradi region, and the three main hospitals in Niamey, Niger, were selected for a one-year prevalence study. Stool samples were collected from all children under 5 having diarrhea with moderate or severe dehydration and tested for the presence of rotavirus using a rapid diagnostic test. Genotyping was performed on a subset of rotavirus positive stools. In addition, a sample of the collected stool was used for bacteriology analyses in Maradi. From December 2009 until December 2010, 5247 children were included in the study. The median age was 9 months [IQR: 7-11 months]. Overall, the proportion of rotavirus positive diarrhea was 26.8% (95% CI: 25.6-28.0) and varied monthly from 10.6% (95% CI: 7.6-13.6) in May to 48.5% (95% CI: 44.7-52.4) in November. Around 65% of the rotavirus cases were children 6 to 12 months old. The most frequent genotypes were G2P[6], G2P[4] and G1P[8]. Coprocultures performed on stools from 1988 children showed *Salmonella* spp. in 10.5% of the cases, *Campylobacter jejuni* in 8.1%, *Shigella* spp. in 3.0%. More than 10% of the *Salmonella* spp. identified carried an extended-spectrum beta-lactamase. This study points out that rotavirus is a major cause of diarrhea with dehydration in Niger, particularly in young children under 1 year of age, and during the cool and dry season. The variety of circulating genotypes in Africa should

be taken into consideration for the development of better adapted vaccines. Bacterial pathogens are also responsible for an important part of diarrhea, and point to the emergence of resistance to third generation cephalosporin.

1406

SYNERGISTIC ACTION OF ROTAVIRUS AND COINFECTING PATHOGENS: EVIDENCE FROM A COMMUNITY-BASED CASE CONTROL STUDY IN NORTHWESTERN ECUADOR

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Diarrheal disease is a leading cause of morbidity in children under five. In developing countries, where diarrheal disease burden is greatest, enteric coinfection is common. There is little understanding, however, of the biological interaction between coinfecting pathogens. We investigated the potential for synergistic action by coinfecting pathogens on diarrhea pathogenesis using an epidemiological framework. We conducted a community-based case control study in 22 villages in northwestern Ecuador. Risk ratios of diarrhea associated with single and coinfections were estimated. Biological interaction of coinfecting pathogens was assessed through the interaction contrast ratio (ICR) and departure of the risk ratios from multiplicativity (MD). After adjusting for age, we found both departure from risk difference additivity and departure from risk ratio multiplicativity in the effects of rotavirus coinfections on acute diarrhea. The ICR was 11.9 (95% CI = 4.7-29.9) for rotavirus-*Giardia* coinfections, and 22.9 (95% CI = 10.5-46.8) for rotavirus-*E. coli/Shigella* coinfections. The MD for these coinfections was 11.8 (95% CI = 3.9-29.4) and 20.1 (95% CI = 2.2-44.2), respectively. This research provides epidemiological evidence for biological synergism between rotavirus and other enteric pathogens. During coinfection, the pathogenic potential of each organism appears to be enhanced.

1407

CREATING A PIPELINE FOR NEXT GEN SEQUENCING OF EBOLAVIRUS ZAIRE

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With Next Generation sequencing becoming a more cost-effective method of sequencing full-length microbial genomes, established pipelines for sequencing pathogens of high impact are greatly needed. These validated pipelines will exponentially increase sequence data available for studies in population dynamics, viral evolution and genetics, and the identification of novel targets for therapeutics, vaccines and rapid diagnostics. Here we developed two methods for full-length sequencing of ebolavirus Zaire using Roche 454 pyrosequencing and Illumina RNA-seq technologies. For 454 sequencing, a "demi-hemi" approach, originally developed at the Broad Institute, was used to design 5-prime amine-modified primers to create long-range PCR amplicons along the entire ebolavirus genome. Amplicons were designed with approximately 500bp overlapping regions to aid in downstream assembly. Illumina sequencing technology was also used to confirm sequence data and further refine primer sequences to capture potential divergent populations within the Zaire strain. While Illumina sequencing provides unbiased sequencing data invaluable to identifying divergent populations, 454 technology can cheaply sequence isolates at a high-throughput capacity with longer sequencing reads that aid *de novo* assembly. Together, these methods have produced a validated 454 sequencing pipeline that has been used to successfully sequence full-length ebola genomes from prepared viral seed stocks and time-course infections of nonhuman primates, with as little as 0.1ng cDNA and over 200x mean coverage depth of the entire genome. Assisted assembly methods using reference genomes, as well as *de novo* assemblies have been created with high success. This method can be used and adapted to sequence many known pathogens for high- and low-throughput sequencing initiatives.

1408

SURVEILLANCE OF MEASLES AND RUBELLA INFECTIONS IN RWANDA: 2003-2011

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Measles and Rubella viruses are still important viral infections in tropical countries including Rwanda. Since 2003, the National Reference Laboratory of Rwanda is accredited by WHO/AFRO as a national Measles laboratory, has been involved in the surveillance of Measles and Rubella infection throughout the country. Cumulative data show that of the approximately 1,894 samples suspected of Measles, 163 were positively identified by ELISA (8.61%). In Rwanda, geographical data indicates that the Rubavu district in the West province, which is at the border with Democratic Republic of Congo is particularly prone to Measles infection. Since 2003, Rwanda has been hit by 3 major epidemics of Measles. The first 2 epidemics, in 2005 and 2006, were limited to the Rubavu district while in 2010, infections were found in various locations, including Kigali city. In collaboration with Uganda Virus Research Institutes, circulating strains were genotyped. It has been observed that in 2005 and 2006, epidemics strains were mostly of the type B2 which is a strain characterized as Congolese. However, during 2010 epidemics, most of the cases were of the type B3, an indigenous strain predominantly found in Burundi. In addition to Measles testing, negative samples were tested for Rubella infection. Of these 1,731 samples tested, 282 were found to be positive (16.29%) and were distributed equally throughout the country.

1409

IMMUNOLOGICAL FEATURES AND PROVIRAL LOAD IN PATIENTS WITH OVERACTIVE BLADDER ASSOCIATED WITH HTLV-1 ARE INDICATORS OF AN EARLY STAGE OF HTLV-1 ASSOCIATED MYELOPATHY

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The majority of HTLV-1 infected subjects are considered as carriers but a high frequency of urinary manifestations of overactive bladder (OB) has been documented in these individuals. The aim of this study was to determine if viral and immunological factors that are associated with development of HTLV-1 associated myelopathy/tropical spastic paraparesis (HAM/TSP) are also observed in patients with overactive bladder associated with HTLV-1. Participants (n=135) were classified as HTLV-1 carriers, HTLV-1 associated overactive bladder (HTLV-1 OB) defined by the criteria of International Continence Society (ICS) and HAM/TSP patients. We demonstrated that peripheral blood mononuclear cells from HTLV-1 OB patients produce higher spontaneous levels of proinflammatory cytokines (IFN-γ, TNF-α and IL-17) than HTLV-1 carriers and similar levels of TNF-α and IL-17 to patients with HAM/TSP. Proviral load was higher in HTLV-1 OB and HAM/TSP than HTLV-1 carriers and correlated positively with production of proinflammatory cytokines. In contrast to HAM/TSP, patients with overactive bladder had serum levels of Th1 chemokines (CXCL-9 and CXCL-10) similar to HTLV-1 carriers and exogenous addition of regulatory cytokines (IL-10 and TGF-β) decreased IFN-γ production in cell cultures from patients with HTLV-1 OB. We conclude that HTLV-1 infected patients with overactive bladder have some immunological features and proviral load profiles in common with HAM/TSP patients. However as they are

still able to down modulate the inflammatory immune response and the recruitment of activated T cells to the central nervous system (CNS) is not enhanced, they present overactive bladder, an oligosymptomatic form of HAM/TSP.

1410

NOVEL VACCINE CANDIDATE PROTECTS MACAQUES AGAINST CHIKUNGUNYA FEVER

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Chikungunya virus (CHIKV) is a mosquito-borne alphavirus that causes an acute febrile illness typically accompanied by rash and severe, often persistent arthralgia. The virus has emerged since 2004 to cause major epidemics in the Indian Ocean, India and Southeast Asia, involving millions of persons. Autochthonous transmission in Italy and France after CHIKV introductions via travelers also underscored the risk that CHIKV poses to the U.S. Because no licensed vaccine exists to protect against chikungunya fever, we used an alphavirus attenuation approach involving a picornavirus internal ribosome entry site (IRES), which replaces the subgenomic promoter, to produce a live vaccine strain capable of inducing protective immunity after a single administration. This vaccine provides robust immunity and protection in murine models, and is incapable of infecting mosquito vectors, an important safety feature for use in nonendemic locations. For further preclinical testing, we subcutaneously or intradermally vaccinated 3 cohorts of 4 cynomolgus macaques, monitored their responses telemetrically to evaluate safety, then challenged them with wild-type CHIKV to assess efficacy. None of the 12 animals developed fever or detectable changes in respiratory or cardiac function after vaccination, and all developed robust neutralizing antibody responses. After challenge, most sham-vaccinated animals developed fever followed by hypothermia, acute viremia, and many also exhibited changes in respiratory and cardiac function. In contrast, all vaccinated animals remained completely normal in all physiological and clinical parameters, and showed no development of viremia from challenge. These results indicate that these new IRES-based vaccine candidates show great promise for use in humans to control chikungunya fever in endemic locations, as well as to reduce the risk of further spread, including into the Americas.

1411

MUTATIONS IN THE E2 GLYCOPROTEIN GENE OF CHIKUNGUNYA VIRUS ASSOCIATED WITH VIRAL-INDUCED ARTHRITIS IN MOUSE MODELS

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Chikungunya virus (CHIKV) is an emerging arbovirus associated with explosive outbreaks of febrile illness often accompanied by rash and arthralgia. The United States Army Medical Research Institute of Infectious Diseases (USAMRIID) developed a live attenuated vaccine virus, 181/clone25 (hereafter 181/25), which despite producing transient arthralgia in a small subset of patients, was shown to be highly efficacious and well tolerated in phase 1 and 2 clinical trials. CHIKV 181/25 was produced by 18 plaque-to-plaque passages of CHIKV strain 15561 in human lung cells (MRC-5). The resulting virus contains 9 nucleotide substitutions, 5 of which result in amino acid changes. In order to probe the specific genetic effects of the mutations, single nucleotide mutation-containing viruses were made that correspond to the amino acid substitutions present in CHIKV 181/25. Two viruses, 7005 and 7014, had amino acid substitutions in the E2 glycoprotein while the other two, 7004 and 7006 had amino acid substitutions in the NSP1 and E1 proteins, respectively. In alpha/beta interferon receptor deficient mice (A129) the two E2 mutation viruses showed a delay in mortality by three days compared to the other two mutants, which showed similar mortality kinetics as wild-type CHIKV, indicating a lack of attenuation. Reduced viremia, pro-inflammatory cytokines, and footpad swelling were also seen in the E2 mutants. In C57bl/6 mice, an immunocompetent arthritis model, the E2 mutants again proved to be more attenuated, behaving similarly to CHIKV 181/25 with less footpad swelling and lower viremias than the E1 and NSP1 mutants. While the single mutations in the E2 gene were not sufficient to create the attenuation seen in CHIKV 181/25, it is clear they resulted in significant attenuation to the parental CHIK virus compared to the mutations in the E1 and NSP1 genes, which resulted in a wild-type-like phenotype. Further studies with viruses containing multiple mutations should be performed to probe the genetic cause of the attenuating phenotype observed in CHIKV 181/25 vaccine virus.

1412

CHALLENGES AND BREAKTHROUGHS IN THE DEVELOPMENT OF SEQUENCING TECHNOLOGY FOR CLINICAL ISOLATES OF LASSA FEVER VIRUS

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Lassa Virus (LV) is the causal agent of Lassa fever, a severe hemorrhagic fever endemic to West Africa. It is responsible for thousands of deaths each year, and evidence suggests that LV acted as a selective agent in recent human evolution. This, combined with observations that it can infect and replicate in its natural host, the rodent *Mastomys natelensis*, without causing severe disease, make it a desirable topic for studies of host-pathogen evolution. In an effort to compile a large dataset of full-length LV genomes, we have applied 454 and Illumina next-generation sequencing technology on clinical samples collected from patients and rodents in West Africa. While we have successfully used both to generate full-length sequences, each platform presents unique challenges and benefits for viral sequencing. 454 technology can generate data from small input volumes, ideal for clinical samples of low viral titers. 454 also produces longer sequence reads, allowing for a wider range of downstream analyses than Illumina-generated sequences. A downside of 454 is that it requires high quality, undegraded starting material - a problem frequently encountered on samples stored under sub-optimal conditions in the field. Illumina sequencing does not require as high quality material but can only be used when other input requirements are met (i.e., low host-contamination, high viral titer). Here we discuss the pros and cons associated with each platform as well as the technical developments that have contributed to their successful application. Our initial results from sequencing have allowed us to make conclusions about new outbreaks of novel LV strains in Northern Sierra Leone, as well as better catalogue circulating strains. Such knowledge will aid in our understanding of viral evolution, allow us to better predict patterns of disease spread, and lay the foundation for better diagnostic development.

1413

MOLECULAR CHARACTERIZATION BY DEEP SEQUENCING OF DIVERSE MEMBERS OF THE GENUS *ORTHOBUNYAVIRUS* FROM MOSQUITOES COLLECTED IN THE AMAZON BASIN OF PERU

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Members of the genus *Orthobunyavirus* (family *Bunyaviridae*) are segmented, negative-sense, single-stranded RNA viruses that are responsible for mild to severe disease in humans. As part of a long-term study of arbovirus ecology in the Amazon basin of Peru, more than 160 viral isolates were made from mosquitoes captured near Iquitos, Peru. Preliminary analysis using immunofluorescent antibody assays (IFA) identified many of these viruses as members of the Group C, Guama, and Bunyamwera serogroups within the genus *Orthobunyavirus*. Follow-up IFAs using complex or virus-specific antisera in complement fixation and hemagglutination-inhibition assays identified some of the viruses as Caraparu, Guama, Itaqui, Mirim, Murututucu, Oriboca, and Wyeomyia viruses; others remain uncharacterized. Additionally, basic knowledge is lacking in regard to the potential reassortment of segments among these viruses in nature. Therefore, we determined the whole genome nucleic acid sequences of these viruses in relation to the viral quasispecies to investigate the extent of intra- and inter-reassortment among RNA segments (S, M, and L) of these viruses. Implications of the reassorted genomes will be discussed.

1414

DEVELOPMENT OF A MULTIPLEX PCR/LDR ASSAY TO DETECT AND GENOTYPE ROTAVIRUS

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Rotavirus and enteric caliciviruses are the most common etiologic agents of diarrhea in children and adults worldwide. The implementation of rotavirus vaccines holds the promise of significantly reducing the associated morbidity and mortality in children. However the vaccines has shown variable efficacy in Africa and Asia attributed to different circulating genotypes than those targeted and/or viral co-infection, which is common in these areas. The WHO has therefore recommended continued surveillance post vaccine implementation. We report the development of a comprehensive molecular assay that can simultaneously detect and genotype the enteric viruses. One-step reverse transcriptase PCR amplifies virus specific targets. Ligase detection reaction and subsequent hybridization of the fluorescent products to beads then detects sapovirus and identifies genotypes of rotavirus, and norovirus. The assay was optimized using previously characterized viral culture supernates and stool specimens and then used to analyze 296 clinical specimens obtained in the US and Ghana. The assay was found to be 97% sensitive and 100% specific with a 100% concordance for genotype determination of norovirus. The rotavirus G- and P-type were determined in 98.6% and 92.3% of the samples, respectively. Mixed genotypes were found in 11.8% of the samples. A significant finding was the identification of two isolates of rare genotypes G6P[6] and G6P[8], both genotypes being reported for the first time in Ghana. The PCR/LDR assay is a sensitive, specific and high-throughput method that can detect rotavirus, sapovirus and norovirus as well as determine the genotype of rotavirus and norovirus. It may therefore be of great utility in epidemiologic surveillance post rotavirus vaccine implementation

1415

HUMAN CELLULAR RESPONSES TO RIFT VALLEY FEVER VIRUS

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Rift Valley Fever (RVF) virus is an arbovirus in the *Bunyaviridae* family that was first isolated in the Rift Valley region of Kenya in the 1930's. The virus is a significant cause of morbidity and mortality in humans associated with devastating epizootic outbreaks in livestock. While endemic to the Rift Valley region, the virus has shown an ability to spread to virgin territory such as Egypt and the Arabian Peninsula. Furthermore, RVFV has emerged as a target for bioterrorism in recent years. RVFV can cause diverse pathology in humans from non-specific viral illness to severe hemorrhagic disease, encephalitis and death. While it is known that RVFV can cause a range of diseases, the pathogenesis is still not well understood. A number of factors such as route of transmission and host immune response are thought to play a role. Animal studies have shown that a type 1 interferon response in the infected individual affects viral clearance. In addition, a delayed IFN response is associated with worse morbidity and mortality. In this study we infect human peripheral blood mononuclear cells (PBMC) with attenuated MP-12 and MP-12 Nss knockout strains of RVFV to determine time to viral uptake and to monitor inflammatory production. Time to viral uptake was determined using plaque assays and qPCR for the L segment of the viral genome and showed that viral uptake happens occurs as early as two hours post infection. The production of various inflammatory mediators, such as TNF alpha and IFN alpha was monitored using ELISA. We show using naïve North American donors that inflammatory responses begin as early as 6 hours after introduction of the virus. Additional studies will elucidate which cells are primarily responsible for the inflammatory response and define innate receptor utilization.

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ECOLOGY OF VENEZUELAN EQUINE ENCEPHALITIS IN THE GULF COAST REGION OF MEXICO

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To characterize the ecology of Venezuelan equine encephalitis virus (VEEV) in endemic regions of the Mexican Gulf Coast, serosurveys in humans and equids, vector incrimination studies, studies of natural infection in equine hosts, and phylogenetic characterization of isolates were conducted in 2008-2010. Human serosurveys from suspected dengue patients (N=237) were VEEV positive in 32 individuals (13.5% seroprevalence), including 5 IgM positives. Equine serosurveys of unvaccinated animals revealed widespread endemic VEEV from the southernmost region in Tabasco State to a northern municipality in Tamaulipas State, located adjacent to the Texas border. Using rodent serosurveys, we identified putative reservoir species of the genera *Sigmodon* and *Oryzomys* in Minatitlan, Veracruz. Using hamster-baited traps in Minatitlan, high-titered mosquito pools (4.9-6.4 $\log_{10}$ PFU/pool) of *Culex (Melanoconion) taeniopus* were identified

in at least 3 transmission events as the likely principal vector of enzootic subtype IE VEEV. As previously observed on the Pacific Coast of Mexico, naturally infected horses developed neurologic disease while producing high-titered viremia (2.4-3.6 $\log_{10}$ PFU). Phylogenetic analysis was performed on isolates from horses, sentinel hamsters, and mosquitoes in Veracruz State. Based on the glycoprotein precursor sequence, all isolates grouped within the Gulf/Caribbean IE genotype and were temporally distinct from the 1963-1969 isolates from the same region. These findings suggest that endemic subtype IE VEEV is currently circulating in widespread regions of the Mexican Gulf Coast, including areas located near the Texas border. Although we implicated *Cx. taeniopus* as the main subtype IE VEEV vector in the Gulf Coast region, *Aedes (Ochlerotatus) taeniorhynchus* mosquitoes were also abundant in this region and have been associated with equine outbreaks on the Pacific Coast of Mexico. Thus, the continuous circulation of endemic VEEV in Mexico has the potential for developing into an outbreak that could rapidly spread into the US via equine amplification.

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RSV, SEASONAL INFLUENZA AND H1N1: CLINICAL MANIFESTATIONS AND CO-INFECTIONS IN 2009

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Respiratory Syncytial Virus (RSV) and Influenza primordially affect children. RSV is a major cause of Bronchiolitis and Pneumonia in children <1 yo, Influenza is characterized by abrupt onset of constitutional and respiratory signs and symptoms. Both manifest in colder months of the year in temperate climates. In April 2009 influenza virus, H1N1 (swine flu) created an outbreak reaching pandemic status in 6 weeks. United States experienced first wave on May 2009 and a second wave, that Puerto Rico also experienced, peaking by end of October. Due to similar clinical presentations of RSV, Seasonal Influenza and Influenza H1N1, physicians differentiated them by Influenza rapid test and RSV nasopharyngeal test. Aim of study: describe clinical and epidemiologic characteristics of RSV, Seasonal Influenza and H1N1. After IRB approval, records of bacteriology and epidemiology from Hospital Episcopal San Lucas were reviewed for positive results of RSV, Influenza, and H1N1 in patients 0y/o - 4y/o, admitted to Pediatrics from October-December 2009 (peak of H1N1 epidemic). Patients with respiratory problems at admission were included, bacterial sepsis was excluded. Records were reviewed for: age, gender, past medical history, symptoms prior to hospitalization, admission or transfer to PICU, supplemental oxygen and days of hospitalization. Forty seven patients tested positive for RSV and/or Influenza. RSV was the most frequently reported (29) with majority of admissions to PICU (5). Seventeen patients had Influenza, 9 H1N1. Most patients with RSV had term birth history while those with Influenza were mostly pre-term. Of 9 patients positive for H1N1 past medical history revealed pre-term birth in 6, and conditions where asthma predominated. One case was co-infected (RSV/Influenza A), admitted with no serious morbidity. Epidemic H1N1 (2009) caused difficulties in differentiation of respiratory illnesses requiring admission. In our experience most children under 4 years had RSV. RSV was associated with most morbidity.

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INTERNAL RIBOSOME ENTRY SITE (IRES)-DRIVEN EXPRESSION OF THE CAPSID PROTEIN IN VENEZUELAN EQUINE ENCEPHALITIS VIRUS INCREASES THE ATTENUATION AND SAFETY OF THE TC-83 VACCINE STRAIN

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The live-attenuated TC-83 strain is the only licensed veterinary vaccine available to protect equids against Venezuelan Equine Encephalitis Virus (VEEV) infection and to protect humans indirectly by preventing equine amplification. VEEV is a mosquito-borne virus endemic to several areas of Central and South America, where both endemic disease and periodic epidemic outbreaks affect hundreds-of-thousands of humans. However, TC-83 vaccine has previously been isolated from mosquitoes collected in the wild. Because it relies on only two point mutations for its attenuation, transmission of revertants represents a major risk to initiate an epidemic or to circulate enzootically. To improve its attenuation and stability, and to prevent infection of mosquitoes, recombinant TC-83 was previously engineered by placing the expression of the viral structural proteins under the control of the Internal Ribosome Entry Site (IRES) of encephalomyocarditis virus (EMCV), which drives translation inefficiently in mosquito cells. However, this vaccine candidate was poorly immunogenic. Here we describe the second generation TC-83 recombinant in which only the capsid protein gene is translated from the IRES, while the viral surface envelope glycoproteins are expressed from a subgenomic message in a cap-dependent manner. This TC-83/IRES/C vaccine does not infect mosquitoes, is stable in its attenuation phenotype after serial passages *in vivo*, and is more attenuated in newborn mice but still protective as well against VEEV challenge. Thus, by using the IRES to modulate TC-83 capsid protein expression, we generated a vaccine candidate that combines efficient immunogenicity and efficacy with lower virulence and a reduced potential for spreading in nature.

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EPIDEMIOLOGICAL MODELING AND RISK ANALYSIS OF VENEZUELAN EQUINE ENCEPHALITIS IN THE HUMAN POPULATION OF COASTAL CHIAPAS, MEXICO IN 2007-2009

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Analysis of 101 febrile illness patients seropositive for Venezuelan equine encephalitis (VEEV) was carried out in a retrospective study along 18 municipalities and endemic VEEV pacific coastal regions of the State of Chiapas in southern Mexico. Geographic information systems (GIS), satellite imagery and a detailed questionnaire were used in the analysis. Using ESRI ArcGIS 10.1 software and spatial statistics tools we measured the geographic distribution of VEEV cases along coastal Chiapas. The distribution of VEE cases were principally located along the Pacific coastal plain with the mean center of positive cases to be in the municipality of Huixtla. The directional distribution of cases around the mean were dispersed in a pattern between the Pacific coastline and coastal mountain range. Temporal and spatial dynamics showed clear separation between cases in the southern and northern regions during the dry season with a peak of positive cases for both regions during the wet season. The analysis was based on Euclidian distances identifying spatially significant clusters

as hot spots. All the spatial analysis was complemented with relative risk (OR) bivariate and multivariate statistical analytical models showing neck stiffness (OR=6.03; 1.2318-29.5801, p=0.027) multivariate; (OR=13.87, IC 3.1861-60.4217, p=0.000), bivariate, muscle weakness (OR=10.12; IC 2.1633-47.4002, p=0.003) multivariate; (OR=23.05, IC5.3134-100.0692, p=0.000) bivariate, taste dysfunction (OR=3.54; IC 1.1380-11.0234, p=0.029) multivariate; (OR=6.40; IC 2.3449-17.5045, p=0.000) bivariate, and photophobia (OR=16.98, IC 2.2321-129.2713, p=0.006) bivariate and conjunctivitis (OR=3.25, IC 1.3273-7.9948, p=0.010) bivariate, as the most important clinical manifestations associated with VEEV for individuals of coastal Chiapas. Overall, our results indicate a coastal band of endemic VEE extending from the Guatemalan border through the State of Chiapas to the adjacent State of Oaxaca.

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THE ROLE OF THE INTERFERONS VERSUS THE ADAPTIVE IMMUNE RESPONSE IN A MOUSE MODEL OF O'NYONG-NYONG VIRUS INFECTION

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O'nyong-nyong virus (ONNV) is an alphavirus transmitted by mosquitoes that shares 90 percent nucleotide sequence identity with Chikungunya virus (CHIKV) and has been the cause of two major epidemics in Africa during the past 20 years. These viruses produce very similar acute febrile illnesses characterized by rash and debilitating arthralgia. While there are many studies into the pathogenesis of CHIKV, little is known about the pathogenesis of ONNV. To determine which portions of the immune system are important in protection during initial infection with ONNV we inoculated several different strains of mice with two different strains of ONNV (SG650 and MP30). These mice included wild type C57BL/6J and mice knocked out for the following genes: STAT1 (STAT1 KO; defective in both type I and type II interferon signaling), Type I interferon receptor (A129), interferon g receptor (IFNgR KO), recombinase activation gene 1 (RAG1 KO). Mice were inoculated subcutaneously with 100-10000 PFU of ONNV or sham inoculated with phosphate buffered saline. Mice were bled to assess viremia, weighed for at least 14 days unless infection was lethal, and observed for illness daily. Tissue samples were collected to test for viral load and for histopathologic evaluation. The C57BL/6J, RAG KO and IFNgR KO mice showed no statistical difference in weight compared to control mice, and never generated detectable viremia. A129 mice demonstrated morbidity but survived. STAT1 KO mice demonstrated an age dependent mortality with 6-week-old mice succumbing to illness by day 12, while 8-14 week old mice developed morbidity as evidenced by weight loss and clinical signs of illness but survived. Tissues of STAT1 KO mice demonstrated a monocytic infiltrate in all major organs and infectious virus was detected in brain and skeletal muscle. We conclude that the adaptive immune system is not necessary for protection against initial ONNV infection in the mouse model while the type I interferon response demonstrates an age-dependent phenotype. These results will be valuable for designing animal models for testing candidate vaccines or therapeutics against ONNV infection.

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SEASONALITY, TIMING, AND CLIMATE DRIVERS OF INFLUENZA ACTIVITY WORLDWIDE

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Influenza is a vaccine preventable disease which annually causes substantial morbidity and mortality, but data on influenza virus activity in tropical countries are limited. We analyzed publicly available influenza data to better understand the global circulation of influenza viruses. We searched for laboratory-confirmed influenza surveillance data in FluNet, Google™, and PubMed using the key words: "influenza," "epidemiology," "season," and "surveillance" to abstract data on the percent of samples testing positive for influenza during each epidemiologic week. The start of influenza season was defined as the first week when the proportion of samples that tested positive remained above the annual median for at least 6 weeks. We assessed changes in the relationship between percent of samples testing positive and average monthly temperature using linear regression models.We identified data on laboratory-confirmed influenza virus infection from 84 countries comprising 5.4 billion (83%) of the world's population. While (44 [94%] of 47) temperate and all four subtropical countries had one annual epidemic, 24 (72%) of 33 tropical countries had one annual influenza epidemic, seven (21%) had biannual epidemics, and 2 (6%) had insufficient data to analyze. Influenza was identified every week in 4 (9%) of 47 temperate, 0 subtropical countries versus, and 10 (30%) of 33 tropical countries (p=0.04). Peak influenza activity occurred within two months after the lowest temperatures in 36 (82%) of 44 temperate, 1 (25%) of 4 subtropical, and 6 (27%) of 22 tropical countries with available data (p<0.001). Influenza activity peaked in Southeast Asia and Oceania during June-July; Australia and China during August; Middle East, North Africa and Mexico during December, Europe and North American during February-March and South America and South Africa during May-June. In conclusion, annual influenza epidemics occur in consistent temporal patterns depending upon climate. Local influenza surveillance and climate data may best inform influenza prevention activities and focus efforts during periods of highest local activity.

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CHARACTERIZATION OF THE NATURAL HISTORY OF LASSA FEVER VIRUS DISEASE IN CYNOMOLGUS MACAQUES FOLLOWING AEROSOL EXPOSURE

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Viral hemorrhagic fever (VHF) in humans is caused by members of four families of enveloped, negative-sense or ambisense RNA viruses; *Arenaviridae*, *Bunyaviridae*, *Filoviridae* and *Flaviviridae*. These viruses are considered possible biothreats due to their potential for airborne and person-to-person transmission, making characterization of the aerosol route of infection paramount. First described in Nigeria in 1969, LASV is endemic to the West African countries of Sierra Leone, Liberia and Guinea. In Africa, members of the *Mastomys* genus of mulimammate rats are persistently infected with LASV from birth. Humans become infected via the respiratory route through exposure to virus in rat excreta, as well as by preparing and eating infected animals. It is estimated LASV may cause 5,000-10,000 fatalities annually; however, there are currently no FDA-approved therapeutics or vaccines. Examining the natural history of

a disease, from exposure through resolution, permits the description of stages of disease course, the immune responses, as well as mechanisms of pathogenesis or correlates of survival. The objectives of this study were to 1) identify early clinical signs that can be used to indicate or predict infection, 2) identify markers that indicate disease progression as well as development of severe disease and 3) determine the impact of anesthesia on clinical disease progression. The natural history of LASV infection following aerosol exposure was examined in the cynomolgus macaque model. Animals were surgically implanted with telemetry providing simultaneous real-time monitoring of pressures, ECG, and temperature as well as daily blood sampling occurred via central venous catheters beginning from Dy -3. Based on these results, a sequential sampling study was designed to collect presymptomatic, early, intermediate and late stages of disease. Summary results will be presented, including hematologic changes (complete blood counts), blood chemistry analysis (Piccolo and iSTAT), coagulation assays, and plasma cytokine responses, as well as pathology findings.

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SUSCEPTIBILITY OF MARMOSETS (*CALLITRIX JACCHUS*) TO MONKEYPOX VIRUS

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Although current nonhuman primate models of monkeypox and smallpox diseases provide some insight into disease pathogenesis, they require a high titer innoculm, use an unnatural route of infection, and/or do not accurately represent the entire disease course. In our studies, we altered half of the test system by using a New World primate species, the common marmoset. Adult male marmosets were intravenously infected with $2.4x10^7$, $9.5x10^5$, and $7.8x10^4$, $5.0x10^3$, 510, and 48 PFU. Clinical, hematological, and viral load data were assessed. Animals were euthanized or succumbed to disease between 6 and 15 days post-infection, in a dose dependent manner. The animals exhibited signs of hemorrhage, had high genome viremia, and altered hematological parameters. At the lower doses, rash was more demarcated and some short-lived macules were observed. As is, our model is 6 logs lower than the current intravenous cynomalgus model and 4-6 logs lower than respiratory models. The aggressive nature of the disease manifested in these animals implicates an even lower dose and warrants exploration of other infection routes. Also, these data should invoke consideration for variola experimentation in marmosets.

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SURVIVAL AND EXPANSION OF HTLV-1-INFECTED CELLS WITH HIGH DNA DAMAGE: RELATIONSHIP BETWEEN SOD1 AND GENOMIC STABILITY

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HTLV1 may exert an initial pro-apoptotic stimulus via the oncoprotein Tax-induced DNA damage. On the other hand, the viral protein Hbz antagonizes some Tax effects and recent studies show that hbz is always expressed in leukemic cells, suggesting its involvement in the maintenance of malignity. Tax has been implicated in the initiation of cellular transformation, chromosomal instability, and induction of cellular DNA damage by reactive oxygen species (ROS). Superoxide dismutase 1 (SOD1) is an antioxidant enzyme present in the cytoplasm, nucleus, and intermembrane space of mitochondria; SOD1 catalyzes the dismutation of superoxide to hydrogen peroxide and molecular oxygen, thus playing an important role in genomic stability. We hypothesize that SOD1 is diminished in cells from HTLV-1-infected subjects with high DNA damage, and that hbz expression is associated with the expansion of these cells. To test this hypothesis we measured Proviral Load (PVL), hbz mRNA, and SOD1 protein levels in cells from HTLV-1-infected subjects with high (HD) and low (LD) DNA damage. Peripheral blood mononuclear cells (PBMCs) were isolated to estimate DNA damage by alkaline comet assay. Two groups of HTLV-1-infected subjects were defined: HD (≥ 51 comets/100 nucleoids; n=18); and LD (≤ 50 comets/100 nucleoids; n=9). We measured PVL and hbz mRNA levels by real time PCR, and plasma SOD1 levels by a sandwich Enzyme-Linked Immunosorbent Assay (ELISA). PVL was expressed as HTLV-1 tax copy number /104 PBMCs. Samples were classified as hbz+ (detectable) or hbz (undetectable). Statistical analyses were based on non-parametric tests. We did not find differences in PVL (p=0.959) or SOD1 (p=0.064) between HD and LD. However, all hbz+ samples came from a cluster of HD subjects with high PVL (3168 ±980), who also showed lower levels of SOD1 than the remaining HD group (p=0.039) and the LD group (p=0.01758). In conclusion, DNA damage is not always associated with low SOD1 levels in HTLV-1-positive subjects. Our results suggest that hbz expression supports survival and expansion of HTLV-1-infected cells bearing DNA damage associated with low SOD1 levels.

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OLIGONUCLEOTIDE MICROARRAYS FOR THE DETECTION AND CONFIRMATION OF ARBOVIRAL PATHOGENS IN THE FIELD

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With the emergence and re-emergence of arthropod-borne diseases throughout the world, it is critical to be able to detect arthropod-transmitted pathogens in a timely manner. Standardized field-diagnostic protocols for identifying arthropod-borne pathogens within any given region of the world are indispensable tools for obtaining real-time information for health-care providers and preventive medicine specialists in field settings. Arboviruses are responsible for major outbreaks of acute, febrile disease throughout most areas of the world. Dengue (DEN), Japanese encephalitis (JE), yellow fever, Chikungunya, and tick-borne encephalitis complex viruses are but a few of the viruses that account for a majority of the arboviral infections that cause morbidity and mortality in humans. Here we report the development and field-testing of an oligonucleotide microarray for the detection and confirmation of arboviruses in pools of field collected mosquitoes. During the field evaluation, mosquito pools were screened by using generic PCR assays and then by using virus specific real-time PCR assays. The phylogenetic relationships among these viruses, their mosquito hosts, and their possible role in causing human disease is also presented.

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MOLECULAR DIAGNOSIS AND ANALYSIS OF IMPORTED CHIKUNGUNYA VIRUS STRAINS, JAPAN, 2006-2010

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Chikungunya (CHIK) virus has re-emerged as an important mosquito-borne pathogen causing epidemics in several parts of the world. The CHIK virus belongs to the Alphavirus genus in the family Togaviridae.

A large-scale epidemic of CHIK fever started in Kenya in 2004 and spread to Reunion Island, other Indian Ocean islands, India, Sri Lanka, Singapore, Thailand and Malaysia. One of the main vectors responsible for transmission between humans is *Aedes albopictus*, which is widely distributed in urban areas of Europe, the USA and East Asia. This fact raises concern that the virus could be introduced and become established in these areas. During 2007-2010, 19 imported CHIK cases were detected in Japan from South and Southeast Asia. The samples were tested for dengue virus as well as CHIK virus by IgM-capture ELISA, real time RT-PCR, virus isolation with Vero and C6/36 cell, and plaque reduction neutralization tests. In this study, we report two cases of imported infection in patients who had returned to Japan from Malaysia and Indonesia. Both viruses were successfully isolated from the cases by using a plaque purification technique. Phylogenetic analysis showed that the strain from Indonesia was grouped into the Asian genotype. However, the isolate from Malaysia was identical to the Central/East/South African genotype and was clustered with currently reported Indian isolates. The strain from Malaysia also had the E1-A226V mutation. These data suggest that the CHIK virus circulating in Malaysia is related to that currently epidemic in South and Southeast Asia. Further characterization of these isolates is in progress.

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THE APPLICATION OF PYROSEQUENCING TO IDENTIFY MULTISPECIES *PLASMODIUM* INFECTIONS IN HUMANS AND APES IN WEST CENTRAL AFRICA

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Recent studies have identified several new species of *Plasmodium* in wild-living chimpanzee and gorilla populations. Using single genome amplification (SGA), we have shown that most apes are co-infected with multiple divergent strains and traced the origin of human *P. falciparum* to western gorillas. This has raised the question whether wild-living apes serve as a recurring source of human infection. While SGA methods are useful in precluding *in vitro* recombination, this approach is not designed to identify low abundance strains in multi-species infections. Here, we describe a novel method of addressing this question by using pyrosequencing technologies. We developed a set of 454 FLX Titanium pan-*Plasmodium* primers that amplify a 510 bp fragment of mitochondrial DNA containing sufficient diversity to differentiate all previously identified ape and human *Plasmodium* species. These primers, in conjunction with the GS FLX System, generate over 1 million reads in a single run, providing both depth of sequencing and high sample throughput. Primers are tagged with sample-specific 12-mer barcodes allowing us to identify the sample origin of each read. To process our data, we have developed a method of rapidly classifying reads by alignment to multiple reference sequences from all known primate *Plasmodium* lineages. Amplicon sequencing by this method has an error rate of $1.5x10^{-3}$ mismatches/nucleotide, low enough to accurately distinguish even the most closely related lineages. In a pilot run of 90 human buffy coat samples from Cameroon, 59 were positive for *P. falciparum* alone, while the remainder represented multiple species infections with *P. falciparum* and *P. malariae* (n=23), *P. falciparum* and *P. ovale* (n=5), or all three species (n=3). Read ratios give an indication of the relative abundance of each *Plasmodium* species in a sample. These data demonstrate that deep sequencing technology can be used to identify mixed-species infections, even when one or more species is present at ratios below 1:2000.

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THE POPULATION STRUCTURE OF AMAZONIAN *PLASMODIUM FALCIPARUM*

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We previously showed that population structure of Peruvian *Plasmodium falciparum* during the peak expansion of malaria in 1990s, following almost two decades of very low malaria transmission, was confined to five major clonal lineages that we referred to as clonets. We have also shown that Venezuelan *P. falciparum* parasites appeared to have somewhat more diverse clonets. Given the shared borders between Brazil and these two countries, we compared the population structure of *P. falciparum* parasites in the Amazon basin, and coastal Peru, using neutral microsatellite markers. The microsatellite data was also combined with drug resistance genotypes and microsatellite markers flanking genes associated with drug resistance (*pfcrt*, *dhfr*, *dhps* and *pfmdr1*) in an aggregate analysis. A total of 190 samples from Brazil were examined from three states (Amapá, Pará, and Rondônia), representing multiple sites and time periods since the 1980s in this study. We analyzed our data using network diagrams, estimates of heterozygosity, and tests for bottlenecks and recent population expansions. Like Peru and Venezuela, Brazil exhibited low parasite diversity and the distance between collection sites was not significantly correlated with increasing genetic differentiation, but unlike these countries there were no obvious clonets. We interpreted the apparent Brazilian *P. falciparum* population structure to be the logical outgrowth of multiple waves of internal migration, admixture, and sexual recombination over past decades. We described the evidence for population bottlenecks at various sites within Brazil. Furthermore, we related the parasite populations from Peru and Venezuela to the Brazilian Amazon basin. At least two of the clonets in the Pervian Amazon were linked with Brazilian Amazon isolates, while the Peruvian coastal lineages were only related by way of samples collected in the Peruvian Amazon. While Venezuelan isolates generally seemed to have more in common with each other, they were also directly linked to Brazilian isolates. This underscored the remarkable clonality of Peru and the intermediate clonality of Venezuela. Our findings suggest that, if Brazilian patterns of internal migration continue in the future, future drug resistance might rapidly spread throughout the country.

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POPULATION GENETICS OF COPY NUMBER VARIATION IN *PLASMODIUM FALCIPARUM*

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Genome rearrangements, such as copy number variation (CNV), are ubiquitous in eukaryotic genomes. Gene dosage changes resulting from gene duplications or deletions may play an important role in adaptive evolution. However, the role of DNA rearrangments has been largely ignored in malaria biology despite the fact that the *Plasmodium* karyotype is highly variable, many rearrangements have been reported in laboratory lines and CNVs are known to influence drug resistance. This project examines extent and functionality of genome rearrangements in the malaria parasite *Plasmodium falciparum*, as well as their origins and evolutionary dynamics. We detected genome-wide CNV in more than 100 parasites from SE Asia, (Cambodia, Lao PDR and Thailand) and Africa (Malawi and Gambia) using comparative genomic hybridization (CGH) on

a custom Nimblegen microarray (the CNV-SNP array) that assays both CNV and SNP variation at high resolution genome-wide. The parasites examined were prescreened to exclude multiple clone infections, and patient derived material was used to avoid artifacts caused by laboratory adaptation. We determined the size, gene content, and population frequency of genome rearrangments within and between parasite populations and evaluate the roles of drift and selection (positive and purifying) in shaping the CNV distributions. Geographical variation in SNPs and CNVs and linkage disequilibrium in regions flanking CNVs were used to better understand the evolution of genome rearrangements in *P. falciparum*.

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HIGH COVERAGE GENOME SEQUENCING OF FIELD ISOLATES PROVIDES UNIQUE INSIGHTS ON *PLASMODIUM VIVAX* BIOLOGY

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The biological diversity of *Plasmodium vivax* is poorly understood, partly because the parasite cannot be easily propagated *in vitro*. As an alternative it is becoming increasingly feasible to characterize the genetic diversity of *P. vivax* isolates across their genomes and to associate genetic variation with biological traits. We present here whole genome sequences generated directly from the blood of three patients and show how robust characterization of the genomic diversity can provide unique insights about *P. vivax* biology. We analyzed blood samples from one Cambodian and two Malagasy patients harboring parasitemias between 0.1 to 0.35% and confirmed *P. vivax* mono-species infection by *Plasmodium* species PCR-based diagnosis. After leukocyte depletion of whole blood (5 ml; using CF11-packed columns), we extracted *P. vivax* DNA from parasitized red cells and prepared libraries from each individual sample after fragmentation of the DNA into 250-300 bp. We sequenced each library on individual lanes of an Illumina HiSeq 2000. We were able to map 20-60 % of the 80 million 100 bp paired-end sequences generated from each sample to the SalI *P. vivax* genome sequence while the remaining reads (40-80%) mapped to the human genome. The very high coverage (100-300X) generated by our sequencing effort provides both a robust description of the genetic diversity across the entire *P. vivax* genome, and allows us to identify and differentiate multiple *P. vivax* strains within each infected patient. In addition, we show that we can analyze variations in sequence coverage along each genome to identify gene duplications and deletions. Finally, we describe multiple DNA sequences shared among the newly sequenced genomes that are missing (for either technical or biological reasons) from the SalI reference genome. Overall, our analyses confirm that sequencing of *P. vivax* genomes from field isolates is very feasible following leukocyte depletion and illustrates that substantial information can be generated regarding genome structure, sequence polymorphism, and complexity of infection.

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GENOME SEQUENCING ASSOCIATION STUDIES: A NEW APPROACH FOR UNDERSTANDING ANTIMALARIAL RESISTANCE IN *PLASMODIUM FALCIPARUM*

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Plasmodium falciparum malaria's rapid adaptation to new drugs allows it to remain one of the most devastating infectious diseases of humans. Understanding the genetic basis of these adaptations is critical to successful intervention. Using next-generation sequencing and drug-sensitivity testing, we performed genome sequencing association studies (GSAS) on 25 recently isolated parasites from Senegal against and 13 antimalarial drugs_including amodiaquine, artemisinin, atovaquone, chloroquine, dihydroartemisnin, halofuganone, halofantrine, lumefantrine, mefloquine, piperaquine, primaquine, pyrimethamine, and quinine_and 20 synthetic compounds. This novel use of whole genome sequence data greatly expands our power to detect associations in low LD populations, as it assays nearly a thousand-fold more positions in the genome than our previous 17,582 SNP array (average coverage of 17.2 Mbp per parasite). However, it comes with some new analysis challenges, including the reliable characterization of marker genotypes from read data (127,362 SNPs and 7,919 microsatellites are polymorphic in this population), handling missing data and more LD in the denser marker set. We adapted recent mixed-model GWAS tools, such as EMMA and GCTA, and selection tools, such as HLR and XP-EHH, to study the heritability of drug response phenotypes and identify known and novel loci associated with drug resistance at genome-wide significance. This demonstrates improvements of the GSAS approach over traditional, array-based GWAS for understanding the genetic basis for antimalarial drug resistance in the wild, potentially identifying important biomarkers for surveillance as elimination and eradication efforts are pursued.

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GENETIC ANALYSIS OF *PLASMODIUM FALCIPARUM* GAMETOCYTOGENESIS

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Within the mammalian host, the *Plasmodium* parasite has two developmental fates: cyclic asexual replication or terminal sexual differentiation (gametocytogenesis). The sexual forms of the parasite (gametocytes) are the only form that is able to survive and propagate in the mosquito vector. Therefore, gametocytes are absolutely essential for parasite transmission. Very little is known about the mechanisms involved in the commitment of *Plasmodium* to sexual differentiation. To gain insight into these mechanisms, we conducted a *piggyBac* transposon-mediated insertional mutagenesis and screened for parasites that no longer formed mature gametocytes. Of 736 parasites (clones) screened in 3 independent transfection experiments, 29 clones did not form gametocytes. For each clone, insertion of *piggyBac* was verified by Southern blot analysis and the disrupted genes were identified by inverse PCR. This led to the identification of 16 putative gametocytogenesis-disrupting genes. Genetic complementation for 4 of the 16 genes was successfully carried out showing that these genes are essential for gametocytogenesis. To epistatically order the 16 genes, we measured their expression pattern along with the expression pattern of other known gametocyte-specific

genes in each of the gametocyte-minus mutants using RT-PCR. We found a subset of the genes that are likely to act very early in commitment to gametocyte differentiation, another subset likely to act just after the committed merozoite invades the red blood cell, and a third set likely to act early (stage I) gametocytes. Thus, we have carried out a comprehensive screen for genes essential to commitment and early differentiation of the *Plasmodium* gametocyte. This line of investigation may lead to novel strategies to reduce parasite transmission and disease burden.

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VARIANT ANTIGEN EXPRESSION IN PEDIATRIC MALARIA

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The ability of *Plasmodium falciparum*-infected erythrocytes to sequester from the circulation into organ microvasculature is associated with much of the lethality of this species. We have investigated genetic variation and antigen expression of parasites in organ biopsies from 25 Malawian paediatric malaria patients. Patients were autopsy-confirmed cerebral malaria cases or parasitaemic controls with an incidental or mild *P. falciparum* infection and another identified cause of death. Cerebral malaria cases had low multiplicity of infection with often a single genetic variant dominating the infection throughout the organs. Expression of the variant surface antigen, *P. falciparum* erythrocyte membrane protein-1, was investigated by quantitative PCR and analysis of expressed sequence tags. Cerebral malaria infections and parasitaemic controls showed similar patterns of antigen expression in host tissues, with particular antigens often being expressed at dominant levels (>33%) by parasites in the brain, heart or gut. These dominant antigens can vary between organ populations within a single host. There was high overlap in the antigens observed in different patients, with 22% of the 644 antigens being detected in multiple patients, and 30% of antigens in the brain also observed in brain biopsies from other patients. This finding was unexpected given the negligible overlap in antigen diversity seen globally and in endemic sites. Our findings suggest that a restricted number of antigens are implicated in sequestration in the paediatric host.

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AN EVALUATION OF THE IMPACT OF INTEGRATED INTERVENTIONS TO IMPROVE ACCESS TO MALARIA TREATMENT IN TANZANIA - THE ACCESS PROGRAM

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The ACCESS Programme was implemented between 2004 and 2008 in two Tanzanian districts to improve access to malaria treatment with a set of integrated interventions at three levels: 1) community level; 2) public heath facilities; and 3) commercial drug sector. The study period saw the switch from Sulphadoxine-Pyrimethamine (SP) to Arthemeter Lumefantrine (ALu) as first line treatment for malaria in 2006. This study aims at evaluating the ACCESS Programme's interventions. We conducted yearly censuses in all health facilities and drug shops between 2004 and 2008 and treatment seeking surveys on approximately 150 individuals in 2004, 2006 and 2008 in the Ifakara Demographic Surveillance Site (DSS). The DSS provided yearly estimates of under-five mortality between 1997 and 2009. Results: We observed improvements in the availability (from 0.24 shops per 1,000 people in 2004 to 0.39 in 2008) and accessibility (from 71% of households within 5 km of a shop in 2004 to 87% in 2008) of drug shops. After the introduction of ALu stock levels of the drug were relatively high in public health facilities (over 80% months in stock), but the drug could only be found in 30% of drug shops. The proportion of children treated with an antimalarial within 24hrs of onset of fever increased from 66% to 89% between 2004 and 2008. However, only 51% were treated with the newly introduced ALu in 2008. Under-five mortality decreased from 28.4 cases per 1000 person years (c/1000py) in the years before 2004 to 18.5 c/1000py in 2008 and 2009. The ACCESS interventions were independently associated with decreases in mortality, controlling for other malaria interventions and contextual factors (incidence rate ratio comparing before 2004 vs. after 2008=0.84, 95%CI=0.72 to 0.99). In conclusion, an integrated approach which tackles both users and providers, recognising the important role of the private retail sector, can lead to improvements in terms of access to malaria treatment and can contribute to decreases in mortality in rural African settings.

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STRENGTHENING MALARIA INFORMATION SYSTEMS IN SOUTH AFRICA: MOVING TOWARDS ELIMINATION

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South Africa has made significant progress in controlling malaria during the past decade. Between the years 2000 to 2010 malaria incidence has been reduced from 8.79 to 0.63 local cases per 1000 population at risk, respectively. Total locally transmitted malaria cases have declined by 92% and malaria deaths have declined by 82% in 2010 as compared to 2000. As a result, South Africa is progressing towards achieving malaria elimination and will need to develop and manage a robust malaria information system (MIS) that will form the basis for effective management by enabling evidence-based decision-making on appropriate use of human, technical and financial resources. To monitor and evaluate progress towards elimination, a comprehensive MIS will need to capture epidemiological information on cases and deaths, GIS mapping of malaria foci and breeding sites and entomological and parasitological data. The purpose of this review is to determine the 2010 baseline for intervention coverage rates stratified among targeted municipalities as well as to determine reporting breakdowns within the current MIS. This paper describes South Africa's National MIS and reviews challenges and best practices in developing an integrated rapid notification malaria database that is unified and standardised across provinces. This standardisation is essential in order to increase information flow and inform programmes of potential outbreaks, allowing for prompt response and investigation. As countries in sub-Saharan Africa move towards elimination, a vigorous surveillance program is necessary to rapidly identify new and reignited foci of transmission, as well as track the movement of vectors, parasites and parasite carriers in this fluid environment. Within the context of South Africa the lack of a functional, standardised information system, and the absence of timely notification of changes in the epidemiological landscape, has led to an inability to inform targeting of interventions due to undetermined foci of transmission. The obstacles encountered and overcome in the restructuring of South Africa's MIS provides necessary guidance to other countries who seek to strengthen control programmes and lay a strong foundation for the eventual development of elimination strategies.

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MALARIA BURDEN AND COVERAGE ESTIMATES FROM THE 2010-2011 MONTHLY 'ROLLING' MALARIA INDICATOR SURVEY (RMIS) IN CHIKHWAWA DISTRICT, MALAWI: A POTENTIAL DISTRICT-LEVEL MALARIA MONITORING AND EVALUATION (M&E) TOOL FOR PROGRAM MANAGERS

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Novel malaria M&E tools are urgently needed to complement the current 'gold standard' Malaria Indicator Surveys (MIS). Rapid up-scaling of malaria control efforts is resulting in substantial reductions in malaria burden across sub-Saharan Africa. As transmission goes down, timely, accurate, sub-national and district level burden estimates are needed to guide increasingly targeted, sub-national control efforts in remaining hotspot areas. To test a novel district level M&E tool, we started a monthly 'rolling' MIS (rMIS) in May 2010 covering 51 villages in Chikhwawa district. This is one of the National Malaria Control Programme (NMCP) focus districts with high insecticide treated net coverage and annual indoor residual spraying with round 1 in January-February 2011. During the first year, approximately 1,200 households were randomly selected using a probability proportional to (village) size. Approximately 100 households were visited each month, and approximately 60 under-fives were tested for anaemia and parasitaemia. Parasitaemic children (by RDT in the field) were treated as per national guidelines. Each month, data was collected in a one-week period by two teams of two people. Data was collected using Personal Digital Assistants, and uploaded daily for quality checking. Results were usually available within two weeks from completion of data collection. Data quality was maintained throughout the whole period. Standard malaria impact indicators (moderate anaemia (Hb<8g/dL) and malaria prevalence in under-fives) as well as intervention coverage indicators will be presented for the first 12 months of the study and by season. The strengths and weaknesses of burden estimates from monthly data collections will be discussed. There will be a particular focus on whether short-term changes in malaria indicators can be detected following district-wide indoor residual spraying after accounting for seasonality. Small-scale, rolling sub-national MIS surveys could be a viable complementary malaria M&E approach for district level program managers and control efforts.

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EVIDENCE FOR LOCAL MALARIA TRANSMISSION IN THE WET SEASON AND IMPORTED MALARIA IN THE DRY SEASON IN ZANZIBAR

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Zanzibar is considering malaria elimination. A critical component of this strategy is better understanding transmission. To investigate malaria transmission in Zanzibar, we combined molecular tools with passive surveillance at all 47 public health facilities in three contiguous districts of Zanzibar from May 2010 to April 2011. Subjects presenting to these facilities with fever who tested positive for malaria by rapid diagnostic test (RDT) or microscopy were enrolled and provided travel history, location of residence, and fingerprick blood samples. We performed genotyping of 9 *P. falciparum* microsatellites to see if genetic information would provide insight into transmission patterns. We enrolled 906 patients, 712 (79%) in the wet season (May-July). 35% of patients presenting in the dry season reported travel outside of Zanzibar in the past month versus 3% of those in the wet season (RR 11, 95%CI 7-17, p <.001), suggesting that a higher fraction of dry season cases may be imported. Thus far, we have 511 complete multilocus genotypes from 384 patients. Closely related parasites (100%, 80-99%, or 50-79% identity) were more likely to come from patients living in the same shehia (smallest administrative unit) than less related parasites, indicating fine-scale spatial clustering of related parasites (RR: 18, 15, and 3 respectively, vs. <50% identity, p<.001 for all). 91 clusters of parasites ≥80% identical were identified, with cluster size ranging from 2 to 19 parasites. In the wet season, 57% of patients were infected with a clustered parasite versus 13% in the dry season (p<.001), demonstrating that local transmission occurs and likely dominates in the wet season. Exploratory visualization of clusters of parasites on a map suggested specific patterns of local transmission. Local transmission appears to account for a high proportion of malaria cases during the wet season in Zanzibar, but imported malaria may play a significant role during the dry season. Analysis of parasite genetics may provide insight into malaria control strategies in low-endemic settings.

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MALARIA ACTIVE INFECTION DETECTION IN AN AREA OF LOW PARASITEMIA PREVALENCE: LUSAKA DISTRICT, ZAMBIA

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Malaria surveillance in Zambia has been via passive case detection. The 2010 Malaria Indicator Survey reported a very low prevalence of malaria in Lusaka and it is suspected that cases of malaria in the urban parts of this district are primarily imported. As transmission rates continue to decline as this area progresses towards malaria elimination, it is necessary to find and treat asymptomatic malaria cases implying the need for cost-effective, focal intervention and surveillance strategies. To address this need, the National Malaria Control Centre, Lusaka District Health Office and partners began malaria active infection detection (AID) where laboratory-confirmed, passively-detected malaria cases are followed-up in the community through testing and treating of households (n=9) immediately surrounding the house of each index case. Since March 2011, community-based teams have led weekly AID responses in 5 health facility catchment areas within Lusaka District with expansion to additional clinics forthcoming. Thus far, a total of 21 index cases have received AID response, with a total of 678 individuals tested for malaria during these responses. Of the 678 tested, 20 (2.9%) were found to be positive. Of the 20, 14 reported a recent (within 1 month) history of malaria indicating their rapid diagnostic test (RDT) results were potential false positives. Remaining positives were likely to be imported as they reported recent travel to malarious areas outside Lusaka. Of the index cases reporting no travel history that were followed-up, there was no indication of ongoing transmission within their household or among sampled surrounding households. Initial results indicate that as low-prevalence areas in Zambia progress towards malaria elimination, it is important to collect patient travel history and to provide public health messaging encouraging individuals to practice

malaria prevention especially when traveling to malaria endemic areas. Furthermore, results indicate AID may be a reasonable, focal intervention for application in low prevalence areas such as urban Lusaka District.

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VALIDATING LAMP FOR IDENTIFYING TRANSMISSION 'HOT SPOTS' FOR MALARIA ELIMINATION AND ERADICATION

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Recent trends in reducing malaria transmission in sub-Saharan Africa and an interest in the steps to reach pre-elimination levels of malaria control mean a paradigm shift in surveillance for malaria is needed. Surveillance and monitoring of all infections including asymptomatic infections is required to find foci of ongoing transmission and to detect when reintroduction of malaria has occurred in an area of local elimination. For this purpose it is likely that a more sensitive test is needed than microscopy or currently available Rapid Diagnostic Tests (RDTs) can deliver. In this study we compare the use of 3 different molecular methods for the purpose of screening blood samples from a large cross sectional survey in a moderate to low transmission setting in north western Tanzania. This study was carried out in Misungwi District, Mwanza, Tanzania, to identify potential *P. falciparum* transmission hotspots by mapping parasite prevalence within house holds. Participants were recruited from 4 villages in Kanyelele namely Mwakalima, Kanyelele, Gambajiga and Budutu. The villages have a total of 33 sub-villages with 1,600 households and a population of about 11,000 people. These villages were characterized for transmission intensity as low transmission with less than 5% parasitaemia and high transmission with more than 10% parasitaemia, using RDTs. A pilot study was conducted of 180 finger prick blood samples collected from participants on Whatman® filter paper, representing 90 randomly selected from each of two villages, one low and one high transmission. Three molecular methods were performed on chelex-extracted DNA samples: LAMP using a mitochondrial target sequence, qPCR with SybrGreen detection, and nested PCR. Taking the nested PCR as the established gold standard, the sensitivity and specificity of LAMP were 85.7% and 91.5%, respectively while the qPCR gave a sensitivity and specificity of 70% and 90%, respectively. In this context of asymptomatic individuals LAMP has performed better than qPCR in terms of sensitivity. The difference in detecting parasite prevalence between LAMP and qPCR was highly significant p=0.0000 (OR 29.7; 95% CI [9.07 - 123.11].These results coupled with the rapidity of obtaining results (<1hour) and the potential ability to perform LAMP in field conditions by direct boiling of blood spots renders this technique a useful tool for identifying hot spots of parasite transmission for malaria elimination.

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MALARIA TOOLS: A USER-FRIENDLY SOFTWARE PACKAGE FOR EXPLORING THE IMPACT OF COMBINATIONS OF INTERVENTIONS IN AFRICAN COUNTRIES

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In the current era of intensified efforts to control or eliminate malaria, individual countries need to prioritise which combinations of interventions they will introduce to reduce transmission and to set realistic expectations about their likely impact. We describe a tool developed for African countries to help guide such decision-making. Underlying the tool is an individual-based dynamic malaria transmission model incorporating current interventions, namely long-lasting insecticide treated nets (LLINs), indoor residual spraying, mass drug administration, IPTi, IPTc and a pre-erythrocytic vaccine. This model has been parameterised by an extensive literature review and Bayesian fitting to multiple data sources. The tool is a downloadable user-friendly interface to the model which can be run on any standard Windows PC (each model scenario running in under a minute). The model includes pre-computed estimates of current parasite prevalence (as a marker of transmission intensity), estimates of LLIN use in the past 10 years, a seasonal pattern of transmission determined by rainfall data and expert-maps of malaria vectors (*An.gambiae* s.s., *An.funestus, An.arabiensis*) at the first administrative level. The user can modify these inputs based on local knowledge and data: if they do, the program will recalculate the natural transmission potential taking account of the fact that conditions are changing both from one year to another and seasonally The user can run scenarios with any combination of interventions, different coverage levels and staggered timings for their introduction. Outputs include projected EIR, parasite prevalence and incidence of clinical malaria over time, which are plotted visually and can be output for further analysis. Thus users can quickly and easily visualise and compare the possible impacts of various control strategies. Future updates will include the ability to enter data on individual program costs so that the cost-effectiveness of different scenarios can be compared.

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EXPULSION OF THE DENGUE VIRUS GENOME BY A PEPTIDE INHIBITOR

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A 33 amino acid peptide (DN59) of identical sequence to the highly conserved amphipathic stem region of the E protein of the dengue 2 virus is able to inhibit DENV entry into multiple cells types with independent entry pathways and at similar low micromolar concentrations. DN59 inhibits entry of all representative flaviviruses tested and is non-toxic to cells. Cryoelectron microscopic images of cell-free, peptide-treated dengue virions suggest that the virions are empty. Three-dimensional reconstructions of these images reveal hollow particles with holes at the five-fold vertices. Peptide treatment of dengue virions in the absence of cellular receptors renders their genomes sensitive to RNase digestion over a wide range of input virus. Western blot analysis suggests that the capsid is still associated with treated virions. Thus, a peptide mimicking a conserved sequence in the membrane-associated stem region of the flavivirus E protein induces expulsion of the viral RNA genome resulting in the inhibition of infection.

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GLUCOSIDASE INHIBITOR AS BROAD-SPECTRUM ANTIVIRAL AGAINST MULTIPLE HEMORRHAGIC FEVER VIRUSES

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Viral Hemorrhagic fever (VHF) designates a group of diseases, caused by enveloped, single-stranded RNA viruses from four different virus families: Arenaviridae, Bunyaviridae, Filoviridae and Flaviviridae. Because many VHF initially do not present with distinguishing symptoms and are difficult to clinically diagnose at early stages, it is important to develop a drug that is universally active against all or most of these agents. Although viruses causing VHF differ in certain features, from the virology point of view, they all have enveloped virions, with viral glycoprotein(s) as envelope. The host ER α-glucosidases are considered to be essential for the maturation, secretion, and function of viral envelope glycoproteins. In this study, we provided genetic evidence that both α-glucosidase I and II are essential host factors for dengue virus and tacaribe virus. Consistent with this notion, we have demonstrated that, imino sugar, the known inhibitor of glucosidases, inhibited multiple hemorrhagic fever viruses from four families (Junin, Dengue, Rift valley fever and Ebola), in tissue culture. Furthermore, treatment with one of our lead imino sugar significantly protected animal death in two mouse models with lethal dengue virus infection. Currently, our extensive SAR study has led to the discovery of several more potent imino sugar derivatives in tissue culture. The *in vivo* efficacy test of these compounds in multiple VHF animal models is underway.

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ECONOMIC IMPACT OF DENGUE ILLNESS AND THE COST-EFFECTIVENESS OF FUTURE VACCINATION PROGRAMS IN SINGAPORE

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Dengue fever and dengue hemorrhagic fever cause 50-100 million cases worldwide and threaten 2.5 billion people in the tropical and subtropical regions. Little is known about the disease burden and economic impact of dengue in resourced countries and the cost-effectiveness of potential dengue vaccines. We estimate the direct and indirect costs of dengue from hospitalized and ambulatory cases in Singapore. We consider *inter alia* the impacts of dengue on the economy using the human-capital and the friction cost methods. Disease burden was estimated using disability-adjusted life years (DALYs) and the cost-effectiveness of a potential vaccine program was evaluated. The average economic impact of dengue illness in Singapore from 2000 to 2009 ranged between US 2010 $0.95 billion and 1.25 billion, with 59-63% corresponding to control costs. We estimated an annual average disease burden of 16-27 DALYs per 100000 habitants, making it comparable to diseases such as meningitis or tuberculosis (22 and 36 DALYs per 100000 habitants, respectively). The rate of symptomatic dengue cases detected by the national surveillance system was estimated to be low, decreasing with age (e.g. ratio of infected symptomatic individuals per detected individual of 1.7-3.8 for 0-24 years, 12.2-50 for >55 years). Potential vaccines are estimated to be highly cost-effective (net savings per DALY averted) for very conservative scenarios, even if vaccination does not reduce the need for vector control. Paediatric vaccination is preferred to mass vaccination for prices per dose greater than $187 and below $365. Mass vaccination, however, presents greater potential of avoided costs and will be preferred for vaccine prices below $187 per dose. If the price of the vaccine is above $45 per dose and below $187, a serology test to administer the vaccine only to non-exposed individuals should complement the mass vaccination program.

1444

DIFFERENTIAL EFFECT IN MONKEYS AND MAN OF PARTIAL IMMUNITY TO THE CYD DENGUE VACCINE, AND IMPLICATIONS FOR THE SAFETY OF TETRAVALENT CYD VACCINATION

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The sanofi pasteur tetravalent dengue vaccine (TDV) candidate is composed of four recombinant, live, attenuated viruses (CYD-1-4). This vaccine, given in a 3-dose, 0-6-12-month regimen, is currently being investigated in clinical phase 3 trials. In a monkey model we previously observed that pre-existing immunity conferred by bivalent CYD vaccination enhances immune responses to subsequent bivalent CYD vaccination performed 2 months later against the remaining two serotypes, without increasing viremia, an indirect indicator of safety. We explored such a complementary, bivalent vaccination regimen as part of a phase 2 clinical trial (Clinicaltrials.gov: NCT00740155): flavivirus-naïve adult volunteers were vaccinated with a bivalent mixture of CYD-1 and -3, and 3.5 months later with a complementary bivalent mixture of CYD-2 and CYD-4. In contrast to findings in monkeys, immunity conferred by CYD-1 and -3 vaccination neutralized the subsequent CYD-2 and CYD-4 vaccine viruses (absent/lower viremia compared to that observed after tetravalent CYD vaccination) and dampened the serotype 2 and 4 specific immune responses. This inhibition was less marked at the serotype-specific cellular immunity level. From these findings we draw two important conclusions. Firstly, as the first bivalent vaccination provided 'protection' against the second (rather than enhancing viremia and immunogenicity) we propose that in the case of natural infection before the completion of the 3-dose TDV vaccination regimen, the partial immunity elicited by the first one or two doses will not enhance disease, even if antibody responses to the infecting serotype are low; furthermore, these findings suggest that heterologous immunity induced by CYD vaccination may be broadly cross-protective between immunizations. Secondly, the interval of time during which cross-neutralization occurs is longer in humans than in monkeys, further highlighting the importance of interspecies differences in terms of immune responses and immunization regimens.

1445

SAFETY EVALUATION AND IMMUNOGENICITY OF FIVE TETRAVALENT ADMIXTURES OF THE NIH LIVE ATTENUATED DENGUE VACCINE CANDIDATES

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Dengue virus (DENV) has become the most important arbovirus worldwide with approximately 36 million cases of dengue fever and more than 2 million cases of severe dengue occurring annually. Because a secondary DENV infection with a serotype different from that which caused the

primary infection is a significant risk factor for DHF/DSS, a DENV vaccine must induce a long-lived immune response to all four DENV serotypes. The goal of the National Institutes of Health (NIH) DENV vaccine program is to produce a minimally reactogenic, highly immunogenic, genetically stable, live attenuated DEN vaccine that is cost-effective and safe for the community. Over the past 10 years, the NIH has tested 8 monovalent vaccines in 15 Phase I clinical trials to identify DENV-1, DENV-2, DENV-3, and DENV-4 candidate vaccine viruses that are safe and maintain the optimal infectivity and immunogenicity profiles for inclusion in a tetravalent formulation. Each monovalent candidate was well tolerated by volunteers with no volunteer experiencing a dengue-like illness. Six monovalent DENV candidate vaccines (a DENV-1 candidate, a DENV-2 candidate, two DENV-3 candidates, and two DENV-4 candidates) were evaluated in five different tetravalent admixtures in healthy adult flavivirus-naïve subjects to identify those admixtures with the most favorable safety and immunogenicity profiles. Safety, infectivity, and immunogenicity data of the five admixtures following administration of a single subcutaneous dose will be presented. Up to 90% of subjects had at least a trivalent antibody response following a single vaccination, with an excellent safety profile similar to that observed in the monovalent trials. Preliminary data from a second dose administered 6 months after the first dose will also be presented. Factors contributing to the immunogenicity profiles of the different admixtures will be discussed.

1446

A RECOMBINANT LIVE ATTENUATED TETRAVALENT DENGUE VACCINE INDUCES NEUTRALIZING ANTIBODIES TO ALL FOUR DENGUE VIRUSES IN HEALTHY ADULT VOLUNTEERS

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The tetravalent live attenuated dengue vaccine (DENVax) is based on the DEN-2 PDK-53 virus. Recombinant DENVax-1, DENVax-3 and DENVax-4 strains were generated in which the prM and E genes of PDK-53 were substituted with those of DEN- 1, -3 or -4 viruses. This approach retains the genetic attenuation markers present in PDK-53. A randomized, placebo-controlled phase 1 clinical trial was performed to evaluate the safety and immunogenicity of tetravalent DENVax formulations in healthy, flavivirus negative adults. The study was completed in Rionegro, Colombia, a high altitude area with no *Aedes aegypti* and no dengue exposure. Low or high dose formulations of DENVax were administered at 0 and 3 months by either intradermal or subcutaneous injection. The vaccine was well-tolerated with mostly mild and transient local or systemic reactions. In addition, DENVax induced significant neutralizing antibody responses to all four dengue viruses after one or two administrations. This study highlights the safety and immunogenicity of the tetravalent DENVax formulations; the vaccine warrants further evaluation in clinical trials in dengue endemic areas.

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PRECLINICAL AND CLINICAL TESTING OF A RECOMBINANT SUBUNIT VACCINE FOR DENGUE

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Dengue viruses are a major cause of morbidity and mortality throughout the tropics and subtropics with an estimated 50-100 million infections annually. To date no specific vaccine or therapy has been licensed to combat this important disease. Live attenuated vaccines for dengue have faced issues with interference between the four viral components. To overcome this issue Merck and Co. is evaluating a tetravalent recombinant subunit vaccine to protect individuals against dengue virus-induced disease. Preclinical studies conducted in mice and non-human primates have demonstrated the immunogenicity and efficacy of both monovalent and tetravalent formulations adjuvanted with alum or ISCOMATRIX™ adjuvant. These studies have shown the capacity of the recombinant proteins to induce balanced tetravalent responses without evidence of interference. Formal preclinical safety assessment studies have demonstrated the acceptable safety of the antigens in various formulations in rats and rabbits. A Phase 1 clinical study of monovalent recombinant protein (DEN1-80E) adjuvanted with alum has been conducted in healthy volunteers and the data from this study will be presented.

1448

MICRODAM CONTRIBUTION TO THE PRODUCTION OF *ANOPHELES* SPP. IN WESTERN, LOWLAND KENYA

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Anopheles funestus is an important vector of human malaria in Africa yet the production of adults of this species from larval habitats is poorly understood. In western Kenya, this species remains an important malaria vector and has been increasing recently in the Asembo Bay region, where insecticide-treated bed nets are in widespread use. One hypothesis for this resurgence is the increased larval habitat provided for *A. funestus* by small dams ("microdams") built in the area. Constructed simply with earthen dikes, the microdams create small reservoirs (mean surface area ~ 0.2 ha) by impounding stream water and rain run-off from upslope in the water sheds of this gently rolling landscape. They are important sources of water for both humans and livestock in the local communities and conserve soil. Naturally growing vegetation, water-logged hoof prints from livestock, and pools of standing water near the microdams create ideal habitats for anopheline larvae. We georeferenced the microdams and used a combination of larval and adult mosquito collections to quantify the contribution of these microdams to the population of malaria vectors in this region. Microdam architecture revealed an up-slope mud plane providing hoof print habitat for *A. gambiae* and *A. arabiensis* larvae, as did the muddy edges of the dams; while vegetated zones provided habitat for *A. funestus* and *A. coustani* larvae. Collections of adult *Anopheles* indoors using the pyrethrum knockdown method showed that houses closer to the microdams had more *A. funestus* than did houses farther away. While the microdams are important sources of water for the communities, they also contribute to the production of malaria vectors in the area, creating a conflict in this rural landscape.

1449

ARE TRENDS IN HOUSING AFFECTING VECTORIAL CAPACITY AND MALARIA TRANSMISSION IN AFRICA?

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Changes in housing design and structure are believed to have made a significant contribution to the elimination of malaria from the USA and Northern Europe. Could something similar happen in Africa? Housing changes are occurring, and not only in urban areas: even in villages that a few years ago contained only mud-and-thatch houses, brick walls and metal or tile roofs are now becoming more and more common. Numerous studies have reported an association between such structural features of house design (screening, ceilings, metal roof) and a reduction in mosquito entry and/or the risk of malaria for the human occupants. Here we review these studies, and discuss the hypothesis that housing improvements may cause a reduction not only in house entry by malaria vectors, but also in vector survival and longevity, and hence vectorial capacity. Such effects could have considerable public health value, because they represent a means by which national authorities could plan to reduce malaria receptivity, as part of a very-long-term elimination strategy. Further studies of such effects are needed in order to give evidence-based advice to householders, local authorities and governments about how best to "build out" malaria.

1450

IMPACTS OF INSECTICIDE-TREATED BEDNETS (ITNS) AND INDOOR RESIDUAL SPRAYING (IRS) ON HOST SELECTION PATTERN BY *ANOPHELES GAMBIAE* S.S., *AN. ARABIENSIS* AND *AN. FUNESTUS* IN WESTERN KENYA

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Malaria continues to be a global public health priority and control interventions are undergoing scale-up of historical proportions. Emphasis has been placed on vector control with insecticides due to its effectiveness, especially when used in nets as insecticide-treated nets (ITNs) and indoor residual spraying (IRS). The WHO has approved vector control using of ITNs and IRS as one of the key malaria control strategies. The use of ITNs and implementation of IRS in selected districts in western Kenya is on the rise, however, mosquitoes are still able to feed and continue to transmit malaria. These interventions may result in marked changes in the vector population structure and behavior, manifested in alteration of biting time and host preference. Knowledge of the biology and behavioral changes of *Anopheles* mosquitoes is important in enhancing understanding of ways of malarial transmission and can further aid in evaluation and designing of appropriate control interventions. A study aimed at determining the actual blood feeding and host preference by *An. gambiae* s.s*., An. arabiensis* and *An. funestus* in the presence of ITNs, IRS or both interventions in western Kenya was conducted in Nyando, Rarieda, Busia and Bungoma districts in western Kenya. Molecular techniques involving Polymerase Chain Reaction (PCR), sequencing and a BLAST search in the GeneBank database were used to test for host blood. In an initial analysis of host blood type in 196 mosquitoes from Asembo in Rarieda district, all samples being *An. arabiensis*, 13.3% had cow blood, 25% human blood, 5% goat blood and 0.5 % rat blood. A number of the analyzed mosquitoes failed either PCR or sequencing, however, the percentages of host blood show a shift in the host selection by *An. arabiensis* with more selections for human blood than cattle in a region with high ITN coverage (above 75%) and high nightly use. Such a shift in the feeding of *An. arabiensis* is of interest since the vector is reported to be highly zoophagic and endophilic. A total of 2000 samples of the three vector species from the remaining study areas are under investigation and results of the study will be presented and discussed.

1451

EVIDENCE FOR NEW MALARIA VECTOR SPECIES IN THE WESTERN KENYAN HIGHLANDS

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In Africa current malaria control interventions such as indoor residual spraying and long-lasting insecticidal bednets rely heavily on targeting the endophilic and late biting nature of a few key mosquito vectors. The presence of vectors that do not conform to these behaviours may limit the effectiveness of such interventions. In a recent entomological study carried out in Kisii, an area prone to malaria epidemics in Western Kenya, the majority of *Anopheles* caught were morphologically distinct from previously described vectors. For 81% of 475 samples caught, no amplification product was obtained following an *An. gambiae* complex species diagnostic PCR. The ITS2 region of ribosomal DNA was successfully sequenced for 395 samples, of which 73% (n= 289) could not be matched to known sequences. The samples were grouped according to similarity of sequences and phylogenetic trees were made. The most abundant group of samples could not be matched to known sequences (173 of 395 samples sequenced) and could not be identified to species level using conventional morphological keys. Sequencing of the mitochondrial DNA CO1 gene also indicated that the majority had no published sequence, and the resulting phylogenetic tree groupings were similar to the rDNA ITS2 trees for the same samples. Of all samples sequenced, 5 were found to be sporozoite positive for *Plasmodium falciparum* by ELISA, all of which were caught outdoors. These 5 had no previously published ITS2 or CO1 region sequence and 3 fell into the most abundant sequence group. 86% of this group (149/173) were caught outdoors and the majority (77%) were caught before 22:30, prior to when people enter their houses. Preliminary morphological identification of house spray catches and larval collections from 2009 and 2011 in 6 other villages across highland Nyanza Province, also revealed the presence of this species. These results indicate the presence of a novel malaria vector in the highlands of Nyanza with early, outdoor biting behaviour. The implications of these findings in relation to malaria control in the area will be discussed.

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COLLAPSE OF *ANOPHELES DARLINGI* POPULATIONS IN SURINAME AFTER INTRODUCTION OF INSECTICIDE-TREATED NETS (ITNS); MALARIA DOWN TO NEAR ELIMINATION LEVEL

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A longitudinal study of malaria vectors, aiming to study *Anopheles darlingi* population dynamics, man-biting and sporozoite rates, was carried out in three villages in the Interior of Suriname between January 2006 and April 2010. During 13,392 man hours of human landing collections, a total of 3,180 female mosquitoes were collected of which 33.7 % were anophelines. *An. darlingi* and *An. nuneztovari* accounted for 99.2 % of the total anophelines collected. The highest mean human biting rate (HBR) observed per survey for *An. darlingi* was 1.43 bites/man/hour outdoors and 1.09 bites/man/hour indoors. Individual ELISA assays of 683 anophelines yielded two *An. darlingi* females infected with *Plasmodium falciparum*. The anopheline HBR decreased to zero in all sites after the onset of malaria intervention activities in 2006, which included the mass

distribution of ITNs. Malaria transmission decreased significantly and Suriname reached the Millennium Development Goal for malaria in 2007. It is concluded that the combination of ITN introduction and climatic events have led to the disappearance of malaria vectors in the study sites in the interior of the country.

1453

DIFFERENCES IN *ANOPHELES GAMBIAE* GENE REGULATION IN RESPONSE TO INGESTION OF LOCAL AND GEOGRAPHICALLY DISTANT ISOLATES OF *PLASMODIUM FALCIPARUM*

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On ingestion of malaria parasites, *Anopheles gambiae* is known to regulate a suite of genes and launch an immune response. Most research on this host-parasite interaction has been done using either model systems or mosquito and parasite strains colonised many years ago. With little research using natural malaria systems the details of host-parasite interactions in the wild remain poorly understood. This study aimed to determine *An. gambiae* gene regulation in recently colonised strains after ingestion of wild *Plasmodium falciparum* isolates. Mosquito strains from Cameroon and Burkina Faso in Central and West Africa were experimentally infected in parallel with their local and distant parasite isolates for comparison. Whole genome microarrays were completed on the mosquitoes 24 hours after infected blood meal ingestion. Eight biological replicates were completed with both the local and distant parasite. Gene regulation was found to be highly diverse across all infections, suggesting that the natural genetic polymorphism present in the samples used here but absent from most previous studies has a great effect on host-parasite interactions, leading to specific gene regulation. Previous work comparing these local and distant infections showed that local mosquito-parasite combinations produce lower infection intensities. The current study highlights a small number of mosquito genes potentially involved in these interactions that are consistently regulated in local infections but not in distant ones. It also shows that the most important factor in determining gene regulation patterns is the parasite population rather than the mosquito strain or local/distant pair. These findings have great impacts on future malaria control methods via the mosquito, especially those which aim to interfere with host-parasite interactions. The fact that these interactions may be differentially evolving over space and or time means that any such control method must be vigorously tested before implementation.

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PLASMODIUM FALCIPARUM DOES AFFECT SURVIVAL OF ITS NATURAL VECTOR *ANOPHELES GAMBIAE*

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The question of the effect of malaria parasites on vector survival has been raised for decades but is still not properly resolved. Several studies addressed the subject but most of them used unnatural *Plasmodium-Anopheles* combinations and therefore disregarded the coevolution between the parasite and the vector. In the most relevant couple for human malaria, *A. gambiae- P. falciparum*, the number of studies is very limited and results are contrasted due to difficulties to obtain large samples of wild-caught infected mosquitoes and to assess their age. In our study, we exposed *A. gambiae* females of a colony from Burkina Faso to *P. falciparum* isolates from naturally infected patients of the same area. An adequate negative control was determined to obtain a corresponding pool of non infected mosquitoes fed on the blood of the same donor. Females exposed and non-exposed to the parasites were maintained until death and dissected immediately after to measure the level of infection. In optimal environment for the mosquitoes, we observed an effect of infection on mosquito survival in interaction with the origin of the blood. This interaction could be the result of variation in parasites (more or less virulent genotypes; single vs. multiple infections), human patients, or less probably in mosquitoes. In conditions of limited nutritional resources after the blood meal, the infected mosquitoes had significantly shorter lifespan than non-infected mosquitoes. This is the first clear evidence of a negative effect of *P. falciparum* sporogony on the survival of its natural vector *A. gambiae*. In malaria transmission, longevity is the most important factor of vectorial capacity. The results have important implications in understanding evolutionary forces that maintain susceptible and resistance alleles to parasite infections in natural mosquito populations. Moreover, the effect of the parasite on mosquito survival might impact malaria control strategies that would aim at increasing mosquito vector refractoriness or specifically target infected mosquitoes.

1455

CHARACTERIZATION OF THE VIRAL FITNESS OF NORTH AMERICAN WEST NILE VIRUS ISOLATES BY *IN VIVO* COMPETITION IN BIRDS AND MOSQUITOES

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West Nile virus (WNV) is a mosquito-transmitted flavivirus of global public, veterinary and wildlife disease importance. Genetic changes in the genome of the invading WNV strain (NY99) have given rise to new seemingly better mosquito-adapted genotypes (WN02) that may represent a key evolutionary mechanism enabling the persistence of WNV. Although some newly emerged WNV genotypes have been genetically characterized, no in-depth study has compared the phenotypic performance and *in vivo* competition fitness of these genotypes in biologically relevant avian and mosquito hosts. Our study addresses the viral fitness of several North American WNV strains utilizing an *in vivo* competition fitness assay in House finches and *Culex tarsalis* mosquitoes. The founding 2003 California WNV isolate belonging to the current dominant WNV genotype (WN02) was genetically marked by site-directed mutagenesis and used as the reference strain for competition against the original invading strain (NY99) and post invasion California isolates. Starting with a 1:1 virus ratio for inoculation, the outcome of competition between the two virus populations was analyzed based on individual replication data resulting from a novel RT-PCR approach, specifically designed for genetic distinction and quantification of mixed virus competition samples. Fitness in birds was determined by examining sera obtained throughout the viremia period and from tissues collected post mortem. In mosquitoes, fitness was evaluated based on the ability of each virus to infect, disseminate and be transmitted after extrinsic incubation. Here, we report a novel diagnostic approach for the quantitative detection of nucleotide polymorphisms, facilitating viral competition studies. Furthermore, we present data on the fitness of North American WNV isolates in their natural hosts and discuss our findings in the context of WNV evolution and persistence.

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TEMPORAL AND SPATIAL FLUCTUATIONS IN ARBOVIRUS MUTANT SWARMS

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Arboviruses often exist within hosts as a swarm of closely related minority genotypes. Although the size and composition of these mutant swarms can have direct phenotypic consequences, the specifics of how stochastic and selective pressures shape arboviral swarms is not fully defined. Although host-specific differences in mutant swarm breadth have been identified with arboviruses, the influence of genetic bottlenecks in mosquitoes during initial virus infection of midgut cells, egress from midgut tissue, salivary gland infection and, ultimately, host transmission remain uncharacterized. In addition to these spatial influences, temporal changes are also important in shaping swarm dynamics both within hosts and throughout seasons. In order to better define swarm dynamics, we've combined experimental studies with genetic analyses of natural isolates and evaluated changes to West Nile virus (WNV) and St. Louis encephalitis virus (SLEV) through time and space. Specifically, studies with WNV in *Cx. pipiens* following feeding on artificial swarms have begun to define within-host bottlenecks. Results indicate that when infecting with equal proportions and high input titers (>8.0 $\log_{10}$ pfu/ml) mutant swarm breadth is maintained spatially throughout the mosquito, yet a significant decline in diversity occurs over time. Results from feeding on different variant ratios indicate also that proportions of variants are maintained in early midgut infection and replication, yet stochastic pressures may result in the dominance of rare minority variants (<2.5%). In addition, analyses of primary mosquito isolates of SLEV from TX and CA indicate a decline in intrahost diversity over time, possibly reflecting the role of seasonal bottlenecks in limiting swarm breath. Studies with WNV isolates from NY are also providing insight into the genetic consequences of local maintenance. These data significantly advance our understanding of how intra- and interhost dynamics alter viral swarms over time and space and how such fluctuations could impact virus evolution and adaptation.

1457

NATURALLY OCCURRING WEST NILE VIRUS DELETION MUTANTS: ROLE AS DEFECTIVE INTERFERING PARTICLES AND INFLUENCE ON PATHOGENESIS *IN VIVO*

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Due to error-prone replication, RNA viruses such as West Nile virus (WNV; Flaviviridae, Flavivirus) exist in individual hosts as heterogeneous populations of related genomes. Genomes that contain deletions are frequently overlooked components of these populations because they are inefficiently detected by conventional approaches to sequencing the virus genome and they are widely considered to be replication-incompetent, dead-end byproducts of infection. We have identified three WNV isolates from birds that have as a portion of their population mutants with large (~2.5 kb), in-frame, internal deletions to their structural coding sequences. To determine whether these mutants function as defective-interfering (DI) genomes, we infected Vero cells at a range of multiplicities of infection (MOI) and evaluated virus output and whether the deletions were maintained through subsequent passage. At high MOIs, deletion mutants persist through several passages and reduced production of full length virus, relative to an infectious clone derived control. Therefore, the mutants appear to act as DI genomes *in vitro*. To determine whether deletion mutants can influence pathogenesis and/or WNV persistence *in vivo*, we infected C3H and C57 Bl/6 mice and day old chickens with virus containing deletion mutant or a full length virus purified from the same isolate, and evaluated morbidity, mortality and virus persistence. These studies allow a clearer understanding of how genomes that contain large in-frame deletions can influence virus replication and pathogenesis, and may shed light on mechanisms for WNV persistence in vertebrates, an emerging health concern in particular regions where the virus is enzootic.

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GENETIC DIVERSIFICATION AND DYNAMICS OF WEST NILE VIRUS IN A NORTHERN TEMPERATE REGION: CONNECTICUT 1999-2008

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West Nile virus (WNV) has become firmly established in northeastern U.S., reemerging every summer since its introduction into North America in 1999. To determine whether WNV overwinters locally or is reseeded annually, we examined the patterns of viral lineage persistence and replacement in Connecticut over 10 consecutive transmission seasons by phylogenetic analysis. In addition, we compared the full protein coding sequence among WNV isolates to search for evidence of convergent and adaptive evolution. Viruses sampled from Connecticut segregated into a number of well-supported subclades by year of isolation with few clades persisting ≥2 years. Similar viral strains were dispersed in different locations across the state and divergent strains appeared within a single location during a single transmission season, implying widespread movement and rapid colonization of virus. Numerous amino acid substitutions arose in the population but only one change, V to A at position 159 of the envelope protein, became permanently fixed. Several instances of parallel evolution were identified in independent lineages, including one amino acid change in the NS4A protein that appears to be positively selected. Our results suggest that annual reemergence of WNV is driven by both reintroduction and local-overwintering of virus. Despite ongoing diversification of WNV, most amino acid variants occurred at low frequencies and were transient in the virus population.

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CHARACTERIZATION OF WEST NILE VIRUSES ISOLATED FROM CAPTIVE AMERICAN FLAMINGOES (*PHOENICOPTERUS RUBER*) IN MEDELLIN, COLOMBIA

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Serum samples were collected from captive otherwise healthy wild birds in the summer of 2008 at the zoological collection in Medellin (Colombia) and tested for the presence of flaviviruses. Total RNA was extracted from sera and tested by reverse transcription-polymerase chain reaction (RT-PCR) using both universal flavivirus and West Nile virus (WNV) specific primers. Eight serum and swab pools from groups of 12-14 wild birds and containing 20 species were evaluated. Interestingly, eighteen samples of twenty five tested from American Flamingoes (Phoenicopterus ruber) were positive for WNV. Selected samples were then inoculated onto subconfluent monolayers of *Aedes albopictus* C3/36 cells and three serially blind passages were conducted before confirmation of virus presence by immunofluorescence. Four isolates (524, 739, 928, and 9835) were further selected for full sequence analysis as well as *in vitro* and *in vivo*

phenotypic characterization. All RT-PCR products revealed West Nile viruses. In addition, sequence analysis showed a total of 15 nucleotide changes resulting in 6 amino acid substitutions in comparison to the WNV New York 1999 strain. Further analysis has confirmed that these viruses are more closely related to Louisiana isolates from 2002. All viruses were highly cytopathic on BHK21 cells while no cytopathic effect was observed on Vero cells. Viruses were highly pathogenic in embryonated chicken eggs and newborn mice. Surprisingly, these isolates diverged in their pathogenicity in 4 week-old Balb/c mice. The epidemiological implications of this new West Nile virus in Colombia and the potential effect on wild and domestic animals as well as human populations are currently being investigated.

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URBAN ECO-EPIDEMIOLOGY OF WEST NILE VIRUS IN ATLANTA, GEORGIA

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Since its introduction in 1999, West Nile Virus (WNV) has become the most important mosquito-borne disease in the USA. WNV activity in the mosquito vectors and reservoir hosts (birds) is clustered in space and time, with transmission focused in certain urban centers (in the East and Midwest) during the summer. However, not all urban areas with intensive enzootic activity see corresponding human cases of disease. In Georgia, substantial WNV presence in the vector and host species has not translated into a large number of human cases, reflecting a similar pattern seen throughout the Southeast, one that is in sharp contrast to some urban areas in the Northeast and Midwest. In a study conducted in Atlanta, Georgia's major urban center, we are addressing the question: in the face of abundant reservoir hosts, disease vectors, and viral presence, why is spillover transmission of WNV (beyond the enzootic) suppressed? We perform comprehensive avian and mosquito sampling in a variety of urban microhabitats, over multiple seasons, to determine the distribution, density, and prevalence of WNV infection in the host and vector species of Atlanta. We focus on sampling in four habitat types within the urban center: mixed-use parks, old-growth forest patches, residential areas, and outdoor animal-holding facilities. Fine-resolution aerial imagery is used to characterize habitat types, percent tree cover, and height of the tree canopy. Avian point counts are conducted at each site to estimate bird species richness and abundance. Using these data, we evaluate the role of Atlanta's diverse urban habitats in disease transmission, focusing on differences in percent tree cover and height of the tree canopy in constraining WNV transmission in time and space. We also explore the extent to which the diversity of avian host species in Atlanta contributes to a WNV "dilution effect." This study targets some of the complex ecological factors governing vector-borne disease transmission in urban settings, combining ecological, epidemiological, and general public health approaches.

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WEST NILE VIRUS (WNV) EPIDEMIOLOGY IN NEW YORK STATE: DO HYDROGEOGRAPHY AND CLIMATE VARIABLES INTERACT IN THE OCCURRENCE OF WNV IN HUMAN CASES AND MOSQUITO POOLS?

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The complex ecology that determines West Nile virus (WNV) epidemiology is not completely understood. The interaction between climate and hydrogeography (HG) in WNV occurrence has not been identified. This study examines the distribution of mosquito pools positive for WNV (WNVm) and human WNV cases (WNVh) across the 62 counties of New York State (NYS) during the 2006 WNV season (May through October). Climate data were obtained from the National Climate Data Center, HG data came from the USGS National Hydrology Dataset, and WNVh and WNVm surveillance data were obtained from the NYS Department of Health. The distributions of total precipitation (PR) during the WNV season, mean July temperature (TM) adjusted for mean temperature standard deviation for May through October, HG, and WNVh and WNVm were mapped in ArcGIS, and specific county clustering was identified using the local Moran's Index (LMI). Poisson regression was used to model the associations between each of the 2 outcomes, WNVh per county population and WNVm per square mile, and the climate and HG variables. WNVh and WNVm were concentrated in the coastal counties corresponding to the Atlantic Ocean/Long Island Sound and Lake Ontario Tributaries watersheds in the southeastern and western counties, respectively. The LMI indicated a high degree of local WNV clustering. The HG parameter, total surface water area (SWA), was an effect-modifier of the association between the presence of WNV and the amount of PR per county. A significant inverse relationship was observed between total PR and WNVh (IRR=0.99; $p<0.0001$) among counties with low SWA (< the median SWA), whereas high SWA (≥ the median SWA) counties showed no association between PR and WNVh (IRR=1.0; $p=0.5$). The same effect-modified associations between PR and WNVm were observed for low (IRR=0.99; $p<0.0001$) and high (IRR=1.0; $p=0.1$) SWA. The associations between TM and WNVh and WNVm were modified in the same way by SWA. These findings suggest the possibility that associations between precipitation and WNV may depend on the surface water present.

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NITRIC OXIDE MEDIATES EXPERIMENTAL HOOKWORM INFECTION

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Hookworm infection is a major cause of anemia, malnutrition, and growth delay in resource poor countries, where more than 500 million people are infected. Human and animal studies confirm that infection with these intestinal nematodes is associated with suppression of the host immune response. We have previously reported higher levels of nitric oxide (NO) from supernatants of spleen cells harvested from *Ancylostoma ceylanicum* infected hamsters compared to uninfected animals. In order to further characterize the role of NO in hookworm pathogenesis and pathology, *in vivo* experiments involving inhibition of NO secretion using N-Monomethyl-L-Arginine (L-NMMA) were conducted. Ten male golden Syrian hamsters were infected with 100 third stage larvae (L3) of the hookworm *A. ceylanicum*. Five infected hamsters received a daily intraperitoneal (IP) injection of L-NMMA starting on day 0 post-infection (PI), while five others had a daily IP injection of PBS. Uninfected control animals received a daily IP injection of either L-NMMA or PBS. At day 36

PI, infected L-NMMA-treated hamsters showed reduced intestinal worm burdens (4 ± 2) compared to PBS-control treated animals (21 ± 4, p < 0.005). Flow cytometry analysis using splenocytes at day 36 PI revealed a higher proportion of $CD4^+$ T cells in infected L-NMMA-treated hamsters than in control animals (12.5 ± 1.2 % vs. 6.8 ± 0.7 %, p= 0.001); a similar difference was also observed for surface IgG^+ B cells (32.0 ± 1.4% vs. 23.1 ± 4.3 %, p= 0.03). There was no difference between uninfected PBS-treated controls and L-NMMA-treated hamsters in the proportion of CD4+ T cells (27.8 ± 1.8 % vs. 27.7 ± 1.8 %) or surface IgG^+ B cells (44.83 ± 1.0 % vs. 46.7 ± 3.1 %). Infected L-NMMA-treated hamsters also had higher blood hemoglobin levels than infected PBS-treated control animals. Together, these data demonstrate that NO modulates both hookworm infection intensity and anemia *in vivo*. Experiments are underway to identify the mechanism(s) by which NO mediates hookworm disease pathogenesis and blunts host cellular immune responses.

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HOOKWORM INFECTION AMONG SCHOOL AGE CHILDREN IN THE KINTAMPO NORTH MUNICIPALITY, GHANA: NUTRITIONAL RISK FACTORS AND RESPONSE TO SINGLE DOSE ALBENDAZOLE TREATMENT

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A cross-sectional study of hookworm infection and household nutrition was conducted in the Kintampo North Municipality, Brong Ahafo Region, Ghana. Children (N=844) between the ages of 6 and 11 years attending 16 schools were screened using anthropometry. Study participants were selected from the tails of a normal distribution of stunting (Height for Age Z-score (HAZ) <= -1.80 or HAZ >= -0.10). Hookworm prevalence at baseline was 39% (109/279), while the overall prevalence of anemia was 62% in the study population. Of children who provided fecal and blood samples (N=248), 35% were co-infected with both hookworm and malaria, 50% were infected with malaria alone, and 3% were infected with hookworm alone. Nearly all (96%) of the hookworm infections were light (<2000 eggs/gram of feces). When controlling for age, gender, household absolute wealth index, and history of recent deworming, statistically significant risk factors for baseline hookworm infection included malaria co-infection (p<0.05), access to health care (p<0.01), lower weekly consumption of protein-rich food groups (p<0.05), and household geographic location (p<0.05). The degree of stunting based on HAZ did not correlate with hookworm infection status at baseline. Hookworm-infected children (N=109) received a single oral dose of albendazole (400mg) and follow-up fecal analysis revealed an overall egg reduction rate of 88% in the study population, with a cure rate of 44%. No single variable collected in this analysis was significantly associated with remaining hookworm positive after receiving a single dose of albendazole. These results confirm prior observations of treatment efficacy for single dose albendazole against hookworm in Kintampo, and offer insight into the potential for current school based deworming programs to impact hookworm disease in areas of moderate prevalence and low infection intensity. Future studies are needed to more clearly define the role of nutritional parameters, including dietary protein intake, in mediating susceptibility to human hookworm infection.

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MATERNAL GEOHELMINTH INFECTIONS INCREASE SUSCEPTIBILITY TO INFECTION IN CHILDREN

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In utero exposure to helminth infections may affect newborn immunity and influence susceptibility to infection during childhood. To test the hypothesis that maternal geohelminth infections increase susceptibility to infection in children, we conducted a nested case-control study in an area of Ecuador where geohelminths are endemic.1004 children with infection data from between 7 months and 3 years of age were selected from an ongoing cohort study. Cases were children with *Ascaris lumbricoides* and/or *Trichuris trichiura*, controls without. Exposure was defined as the presence of maternal infection with *A. lumbricoides* or *T. trichiura* detected in a stool sample collected in the third trimester of pregnancy. To control for risk of infection, the study was restricted to households with at least one family member infected with *A. lumbricoides* and/or with T. trichiura, as determined by stool samples collected after the child's birth. Children of mothers with geohelminth infections had a significantly greater risk of infection relative to children of uninfected mothers (46.3% exposed vs. 30.7% unexposed, adjusted OR 2.62, 95% CI: 1.90-3.62, p<0.001). This effect was particularly strong in children of co-infected mothers (62.6% exposed vs. 30.7% unexposed, adjusted OR: 5.57, 95% CI: 3.46-8.98, p<0.001). No significant differences were observed between intensities of infection or between distributions of egg counts. Our data suggest that maternal geohelminth infections increase susceptibility to infection during early childhood. This may be due to *in utero* immune modulation by maternal geohelminth infections that induce greater neonatal and childhood tolerance.

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IMMUNE REGULATION BY TREG SUBSETS DURING HUMAN GEOHELMINTH INFECTION: EFFECT OF ANTI-HELMINTH TREATMENT

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Chronic helminth infections induce profound regulatory immune responses, leading to strong T cell hyporesponsiveness. The reduced immune reactivity does not only affect parasite antigens, but may also be extended to vaccines or to other coinciding pathogens, such as malaria parasites. Helminths induce regulatory T (Treg) cells, characterized by high expression of CD25 and FOXP3, which are able to downregulate effector T cell responses. The question arises whether Tregs are responsible for the immunosuppressive state during human helminth infection and might have consequences on bystander responses. To investigate Tregs in helminth infections, we set up a study in a rural area on Flores island, Indonesia where schoolchildren with or without helminth infections were selected. $CD4CD25^{hi}$ T cells were magnetically depleted from PBMC and subsequently, mock- and Treg-depleted PBMC were cultured with BCG, *Plasmodium falciparum* parasitized RBC (pRBC) or control RBC. Proliferation and cytokine responses were analyzed as function of helminth infection status. Proliferation to BCG and pRBC was reduced in geohelminth-infected compared to uninfected children. This difference was not reflected in changes in Treg frequencies in peripheral blood. However, following $CD4CD25^{hi}$ T cell depletion proliferation in the

helminth-infected group was restored to levels similar to those seen in helminth negatives. Removal of $CD4CD25^{hi}$ T cells also increased the IFN-γ production in response to BCG and pRBC only in helminth positive children. Although numbers of Treg were not increased, Tregs displayed a higher capacity to suppress proliferation and IFN- γ production to bystander antigens in geohelminth-infected compared to uninfected children. In an ongoing anti-helminth treatment trial, we are investigating Treg phenotype and function in a larger cohort at Flores island. Treg depletion assays were carried out and repeated 1 and 2 years after anti-helminth treatment, enabling us to see differences in helminth-infected, -uninfected and -treated individuals. Cytokine and proliferation data from the longitudinal Treg study are still under analysis, but this is expected to be finalized by September 2011. Treg depletion in human helminth infection is a new concept and this helminth-induced effect on the immune system may have major consequences in vaccine implementation and malaria elimination strategies.

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INCREASED LOCAL REGULATORY T CELLS AND DIMINISHED IGE EXPRESSION IN DUODENAL MUCOSA OF SS/HTLV-1 COINFECTED PATIENTS

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Strongyloides stercoralis (SS) is an intestinal nematode unique in its ability to replicate in the human host, permitting ongoing cycles of autoinfection, persisting for decades within host. Although usually asymptomatic, overwhelming infections can occur in SS and HTLV-1 co-infected individuals (SS/HTLV-1). Regulatory T cells (Treg) are able to blunt specific Th2 response necessary to control the parasite. We previously reported that peripheral blood Treg are increased in SS/HTLV-1 and correlate with low Th2 responses. We hypothesized that local Tregs are increased in duodenal mucosa of SS/HTLV-1 patients. Paraffin embedded duodenal biopsies were obtained from 10 HTLV-1/SS subjects and 3 control samples from non-parasitic chronic duodenitis (NPCD) subjects. Immunohistochemistry was performed with CD3, IgE and FoxP3 human mAbs. The numbers of cells were counted using a conventional light microscope. 400x magnification images were taken and the area was measured using the ImagePro Plus software. The numbers of CD3+, FOXP3+ and IgE positive cells per 0.35 mm^2 were assessed. Patients with HTLV-1/SS had higher T lymphocyte counts in areas non-adjacent to the parasite (NAP) compared to areas adjacent to the parasite (AP) (NAP [P50]: 24.5 IQR: 20.3-36.3, AP [P50]: 6.5 IQR: 2.8-12.3, p=0.0003 Mann-Whitney). The number of IgE expressing cells was higher in areas non-adjacent to the parasite (NAP [P50]: 35 IQR: 17.5-57.5, AP [P50]: 3 IQR: 0.5-6.0, p=0.001 Mann-Whitney). Patients with HTLV-1/SS show an increased counts of Tregs cells (FoxP3+ expressing cells) when compared with patients with non-parasitic chronic duodenitis (SS/HTLV-1 [P50]: 3.3 IQR: 0.6-8.4, NPCD [P50]: 0.0 range 0-1.2, p=0.099 Mann-Whitney). In conclusion, our data shows an increased Treg cell count in the duodenum of SS/HTLV-1 patients. In addition, T lymphocytes and IgE expressing cells were diminished in parasite adjacent areas. Altogether, this study suggests an important role for Tregs in down-regulating local parasite effector responses during SS/HTLV-1 co-infection.

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SEROPREVALENCE OF ANTIBODIES TO *STRONGYLOIDES STERCORALIS* NIE AS A TOOL TO IDENTIFY COMMUNITIES FOR ANTHELMINTIC INTERVENTIONS IN NORTHERN ARGENTINA

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Infections with *Strongyloides stercoralis* (Ss) are cosmopolitan and mostly subclinical; new diagnostic approaches are needed to define the prevalence and distribution of infections and to monitor intervention activities. The identification of Ss-specific recombinant antigens increases the availability of serologic assays for these efforts. We evaluated the performance of Ss-NIE-1 by ELISA previously shown to be sensitive and specific in the setting of a mass deworming program in a rural community of northwestern Argentina (S 22°53´60´´; W64°20´06´´) with a total population of 618 people. Prior to drug administration for community-wide treatment, a subset of individuals was randomly selected for soil transmitted helminth (STH) assessment at baseline evaluation. Of the 80 persons (mean±SD age: 28.5± 20) who participated in the Ss study, 48 (mean±SD age: 30.5± 21) were also evaluated by stool analysis that included 4 techniques (concentration-sedimentation, agar plate, Harada-Mori and Baermann with bone charcoal culture). Serum samples were analyzed with the Ss-NIE-1 ELISA. The results demonstrated Ss larvae in 16% (8/48) stool samples; in contrast, 31% (25/80) were positive by serology (mean±SD age: 32.8.5± 20.8). Thus, there was a significant difference between the stool and more sensitive serum analysis ($p<0.05$ by Fisher's exact test). Although three stool positive individuals (mean±SD age: 31.3± 22.4) were Ss-NIE seronegative, the known improved sensitivity of the SS-NIE ELISA for Ss infection with its higher throughput and ease of performance provides an epidemiologic tool to identify Ss-endemic regions of the world in need of anthelmintic therapy. Moreover, the Ss-NIE ELISA confirmed the high prevalence of this STH in the study area, which aids in region-wide strategies for community intervention for this important STH.

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STAGE-SPECIFIC GENE EXPRESSION BY *STRONGYLOIDES STERCORALIS* FOLLOWING HOST INVASION: A MICROARRAY-BASED ANALYSIS

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The key molecular factors that enable *Strongyloides stercoralis* (Ss) larvae to initiate infection have not been elucidated to date, and are critical for developing new insights into the biology of this nematode and identifying novel drug and vaccine targets. We describe here a microarray analysis of gene expression by infective third stage larvae before (L3i) and 72 hours after (L3+) host invasion. Differentially labeled cDNA obtained from RNA extracted from these larvae was hybridized to a Ss DNA microarray. Genes that were more highly expressed in either stage were identified based on a cutoff of 2 fold increased gene expression and adjusted p-value < 0.01 [false discovery rate of 1%]. Using this method, 96 differentially expressed genes were identified. Expression of genes putatively encoding extracellular matrix proteins, such as collagen, was notably increased in L3i compared to L3+ larvae (p = 0.0003). By contrast, an enzyme homologous

to *Brugia malayi* chitinase (BLAST E value 4E - 009) was significantly expressed in the L3+ stage (p = 0.001). Following invasion, genes putatively encoding components of the ubiquitin proteasome pathway were highly expressed. L3+ larvae additionally demonstrated a significant increase in the number of differentially expressed genes encoding enzymes with putative catalytic activity (such as hydrolases, ligases and transferases ; p = 0.02). Stage-specific differences in metabolism were found. A significantly higher number of differentially expressed L3i genes were putatively involved in energy metabolism (p = 0.042), while twice as many differentially expressed L3+ genes were putatively involved in amino acid, carbohydrate and lipid metabolism. These data indicate that Ss larvae downregulate expression of extracellular matrix and energy metabolism genes, and increase expression of genes encoding catalytic enzymes following host invasion, and provide important clues regarding differentiation to the L3+ stage and establishment of infection following host invasion.

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THE IMPACT OF A COMMUNICATION CAMPAIGN ON USE OF ORAL REHYDRATION SOLUTION BY MOTHERS OF CHILDREN UNDER FIVE IN MALAWI

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The use of oral rehydration solution (ORS) has stagnated at 40% in most countries since 1995. Population Services International (PSI) began a social marketing program in 2005 in Malawi to improve access to and use of *Thanzi* ORS for children under five. PSI uses targeted communications to bring about behavior change in early recognition and treatment seeking during diarrheal illness. Repeated household surveys were used to evaluate the impact of the communications campaign. Nationally representative household surveys were conducted in 2005 and 2008, and are complemented by 2010 preliminary DHS results. Guided by a conceptual framework for understanding caregiver behavior, multi-item scales were developed to measure opportunity, motivation and ability factors. Factor and reliability analysis guided scale development. At baseline, multivariate logistic regression was used to test for adjusted associations between hypothesized behavioral determinants and diarrhea treatment. At follow-up, significant changes in behavior and behavioral determinants were examined. At baseline (2005), perceived availability of ORS and knowledge of causes of diarrhea were found to be key determinants that differentiated users of ORS from non-users. Baseline results guided development of a communication campaign involving mass media and interpersonal communication. In 2008, perceived availability, knowledge, and self efficacy improved significantly. Similarly, attitudes toward the *Thanzi* brand improved. In 2005, 58.1% of children with diarrhea in the past two weeks were reported to have taken ORS. This increased to 64.3% in 2008 and to 69% in 2010. *Thanzi* ORS made up 75% and 83.7% of the ORS utilized in 2005 and 2008 respectively. Although a number of factors could have resulted in the increased use of ORS to treat diarrhea in under-five children in Malawi, *Thanzi's* proportionally large ORS market share, marketed by PSI using a social and behavior change approach, is indicative of the role that evidence-based social marketing interventions can play in improving diarrhea treatment.

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DO LIMES ACCELERATE SOLAR DISINFECTION OF WATER (SODIS)? A LAB/FIELD STUDY COMPARING EFFICACY WITH MOUSE NOROVIRUS, *E. COLI* AND MS2 BACTERIOPHAGE

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Two million people in the world die annually of diarrheal disease, mostly among the 850 million people who do not have access to sources of improved drinking water. Household water treatment (HWT) can prevent waterborne illness. Solar disinfection of water (SODIS) is a HWT method that involves exposing water bottles to sunlight for 6 hours. A potential mechanism to increase use of SODIS would be to reduce the treatment time. Psoralens and acids both interact synergistically with ultraviolet radiation to accelerate inactivation of microbes. This study replicated field-based SODIS conditions by using 2L bottles and dechlorinated tap water in solar experiments (n=8). SODIS + lime juice bottles contained approximately one-half lime in 2L water. 5-Methoxypsoralen was used as a control for naturally occurring psoralens. Treatment efficacy (n=3) was evaluated for E. coli, MS2 bacteriophage, and mouse norovirus. E. coli was ablated >6.08log by SODIS + lime slurry, >5.98log by SODIS + lemon juice, and 5.59log by SODIS + lime juice in 30-minute solar exposures, compared with 1.50log for SODIS alone. MS2 was inactivated >5.77log by SODIS + lime slurry, 3.10log by SODIS + lime juice, and 2.73log by SODIS alone in the sunniest of three 2.5-hour solar exposures. In contrast, mouse norovirus, a surrogate for human norovirus, was highly resistant to all forms of SODIS. Using simulated sunlight in laboratory ultraviolet experiments, SODIS + lime slurry and SODIS + lime juice inactivated mouse norovirus 2.03log and 1.84logs, respectively, after 6 hours lamp exposure, while conventional SODIS achieved only a 0.30log reduction. In field-based conditions, all treatment bottles (n=3) showed <2log reductions in mouse norovirus after a 6-hour solar exposure. A pH of <4 facilitated inactivation of E. coli, while psoralens were more effective than pH in inactivating viruses. SODIS + citrus dramatically reduced E. coli levels in just thirty minutes, a treatment time on par with boiling. Furthermore, familiarity with citrus juice may make this method appealing to potential users. Mouse norovirus, a human norovirus surrogate, was highly resistant to all SODIS treatments. The efficacy of SODIS against human norovirus should be investigated further.

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AN ACCEPTABILITY AND FEASIBILITY PILOT OF HOUSEHOLD LEVEL WATER TREATMENT IN URBAN BANGLADESH

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Household water treatment prevents diarrhea. However, most household water treatment interventions fail to be used consistently. We conducted a small trial in urban Bangladesh to assess the acceptability of various approaches for point of use water treatment. In May and June 2010, we used the trial of improved practices methodology in 15 low income compounds with a total of 88 individual households. Of 3 available water treatment options, we introduced and provided 1 option in each compound for a free 30 day trial. These options included liquid sodium hypochlorite from a dispenser for communal use (n=58), liquid sodium hypochlorite from a dropper bottle for household use (n=20), and a double chamber ceramic filter for household use (n=10). For the 78 households that received chlorine-based options, we provided

standardized water containers to 51households. In each of 5 follow-up visits we conducted either semi structured interviews, household observations, and/or group discussions. The double chamber ceramic filter had the most (10/10), and the chlorine dispenser had the least (10/58) sustained self reported use. Most of the participants were able to follow the operating instructions as observed during follow up visits. However, 25/27 participants without the standardized water container compared with only 5/51 participants with the standardized container had difficulty achieving the correct concentration of chlorine. Frequently mentioned benefits of using the various technologies included ease of use, the clarity of treated water and being less time consuming than boiling water. Barriers in using the technologies included the strong smell of chlorine, lack of a standardized storage container with the chlorination options, and the leaking chlorine dispenser's valve. The double chamber water filter was most acceptable because the filtered water did not have any odor, whereas, water chlorination was less acceptable due to the strong smell of chlorine. Odor minimizing techniques, including better models of dispenser that do not leak and standardized containers should be tested. Prospective users should be reassured that the odor is inconsequential to their health and well-being. In addition, the reported benefits, such as clarity of treated water compared to supply water and the short treatment time required can be used to promote sustainable water treatment behavior change interventions.

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THE RISK OF MODERATE AND SEVERE DIARRHEA IN CHILDREN LESS THAN FIVE YEARS OLD IS INCREASED AMONG FAMILIES WHO SHARE A SANITATION FACILITY

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The WHO classifies sanitation facilities shared by two or more families as "unimproved" for tracking progress towards the Millennium Development Goals. However, little is known about the health impact of shared sanitation. We examined associations between household sanitation facilities and moderate to severe diarrhea (MSD) among children <5 years old participating in the Global Enteric Multicenter Study (GEMS) in 7 developing country sites in sub-Saharan Africa and South Asia. Cases seeking care for MSD (defined as ≥3 loose stools in 24 hrs with one or more of the following: sunken eyes, skin tenting, dysentery, IV rehydration, or hospitalization) were enrolled at health facilities. Age-, gender- and community-matched controls were enrolled at home. Sanitation facilities were observed during follow-up household visits (between 60-90 days) to all GEMS participants. Among 8,977 cases and 12,278 matched controls, 5.7% practiced open defecation; the remainder used pit latrines (55.5%), VIP latrines (3.6%), pour flush toilets (32.4%) and flush toilets (1.6%). No type of sanitation facility was statistically associated with MSD overall or at any site. However, families of case children more commonly used shared sanitation facilities than control families (47.5% vs. 41.2%, mOR = 1.2; 95% CI: 1.1-1.3), overall and in Pakistan (mOR=1.7; 1.4-2.0), Mali (mOR=1.2; 1.1-1.4), India (mOR=1.3; 1.0-1.6), and Kenya (mOR=1.2; 1.0-1.5). The odds of MSD for shared sanitation were increased two-fold if feces was present (mOR=2.2; 1.6-3.2) than if was absent (mOR=1.2; 1.1-1.3). While access to unshared sanitation facilities was more common among higher-income households, shared sanitation facilities were consistently more common among case than among control households across all wealth index quintiles. Our observations indicate that shared sanitation facilities can increase the risk of diarrhea, regardless of the type of facility, and supports their classification as "unimproved". Increasing access to private sanitation facilities may reduce diarrhea incidence among young children.

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ACCESS TO WATERLESS HAND SANITIZER IMPROVES HAND CLEANING BEHAVIOR AFTER TOILET USE AT PRIMARY SCHOOLS IN KIBERA, KENYA

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Hundreds of millions of school days are estimated to be lost each year due to diarrheal illness. Handwashing is an established effective strategy to reduce diarrheal and respiratory illnesses. However, promoting handwashing is challenging in settings with limited water access. This study investigated the impact of providing waterless hand sanitizer on student hand cleaning behavior and health in six primary schools within Kibera, Kenya. Two schools received a waterless hand sanitizer (HS) intervention, two received a handwashing with soap (HW) intervention, and two received no intervention (controls). Hand cleaning behavior was monitored for 8 weeks through structured observation and by surveillance cameras placed at school toilets. Students were interviewed weekly to monitor diarrheal and respiratory illness. The average rate of hand cleaning after toileting was 82% of 2589 events observed in HS schools (OR=8.1, 95% CI=2.8-23.0), 38% of 3607 events observed in HW schools (OR=1.1, 95% CI=0.2-7.5), and 37% of 3031 events observed in control schools. At HW schools, soap was used for 97% of handwashing events, while at control schools, soap was used for only 6% of events. Water was unavailable in 39% and 30% of observations at HW and control schools, respectively. When water was available, handwashing rates were 62% at HW schools and 53% control schools. Students at HS schools were 26% less likely to report diarrhea (p=0.005), 20% less likely to report cough (p=0.013), and 30% less likely to have a runny nose observed by enumerators (p=0.009) compared to control schools. Students at HW schools were 19% less likely to report diarrhea (p=0.04) and 32% less likely to have an observed runny nose (p<0.001) compared to control schools. Among Kenyan primary schools, provision of waterless hand sanitizer markedly increased rates of hand cleaning after toilet use, while provision of soap and water tanks did not. These findings suggest that use of waterless hand sanitizer is a promising option for reducing infectious disease in schools with limited water access.

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ASSOCIATIONS WITH HANDWASHING IN THE HOME AND RESPIRATORY AND DIARRHEAL ILLNESS IN CHILDREN UNDER FIVE YEARS OLD IN RURAL WESTERN KENYA

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Diarrhea and pneumonia are leading causes of death in children worldwide. Handwashing with soap is effective in reducing childhood diarrhea and pneumonia in resource poor areas in south Asia, but data are sparse for Sub-Saharan Africa. We observed presence of a designated handwashing (HW) location, soap at a designated HW location, or soap in the home, and examined the association with longitudinal prevalence of

diarrhea and acute respiratory illness (ARI) in children <5 years old in rural Asembo, Kenya. Syndromic surveillance for diarrhea and ARI in children <5 years old is conducted biweekly in Asembo. In April 2009, we assessed households in the surveillance area for handwashing indicators. Syndrome data collected in the four months preceding the survey was used. We used generalized linear regression models to estimate differences in longitudinal prevalence of illness associated with living in households with each HW indicator, adjusted for household wealth, age, sex, and within household intraclass correlations. The sample included 2547 children in 1745 households. Overall longitudinal prevalence of diarrhea and ARI were 2.5 and 12.7 days of illness per 100 child-days, respectively. Longitudinal prevalence was 30.7% lower (95% CI 24.4%, 34.4%) for diarrhea and 20.5% lower for ARI (95% CI 12.2%, 25.8%) in children in households with observed soap compared to children in households with no observed soap. A designated HW location was identified in 3.3% of households, and 1.2% had a designated HW location with observed soap and/or water present, and neither was associated with a difference in diarrhea or ARI prevalence after adjustment. The presence of observed soap in the home, a proxy measure of handwashing behavior, is associated with reduced illness in children in rural western Kenya. A minority of households had a designated HW location, which, based on previous studies of handwashing behavior, offers substantial opportunity to increase handwashing behavior by providing a visual cue to stimulate handwashing at critical times for pathogen transmission.

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THE ASSOCIATION OF SOAP IN HOUSEHOLD ON CHILD MORTALITY FROM RESPIRATORY INFECTIONS

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Respiratory disease is the leading cause of childhood death globally. Hand washing with soap prevents respiratory disease morbidity but there is no direct evidence of its impact in preventing deaths from respiratory infection. We explored the contribution of household handwashing behavior to child mortality from respiratory diseases in rural Bangladesh. We obtained data on deaths among children <5 years of age from a large scale, sanitation, hygiene education and water supply intervention in rural Bangladesh which spanned 16 districts. To assess cause of death, we conducted verbal autopsies approximately 1 year after child death. Each death among a child <5 years (ICD-9 code 869.3-869.6) was age matched to three children from the same community who did not have respiratory symptoms at the time of interview. We interviewed family members, conducted 5 hours of household structured observation including observation for the presence of soap in the household. We constructed a socioeconomic index using principal component analysis of parental education, household assets, number of rooms, and housing material. We used logistic regression to adjust for immunization, socio-economic status, family size, and number of people sleeping in the same bed as child. Compared to 315 age matched healthy children, the 105 cases were more likely to have smaller families (p<0.001) and less educated mothers (p=0.012). Cases (29%) were more frequently assigned to the poorest wealth quintile than controls (18%). In multivariate analysis, having had any childhood vaccination [AOR: 0.26, CI: 0.11-0.58] and having any bar soap in the home [AOR: 0.53, CI: 0.29-0.98] was independently protective. Household participants washed hands in only 10 (2%) of 523 observed respiratory events such as coughing, sneezing, or nose cleaning or blowing. All 10 washed with only water. Household ownership of soap, a proxy hand washing measure was associated with deaths from respiratory disease. However, handwashing with soap was not practiced after contact with respiratory secretions. Understanding optimal strategies within households to interrupt respiratory pathogen transmission by hand washing would help decrease respiratory disease mortality as well as morbidity.

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FILARIAL-EXPANDED HUMAN NATURAL REGULATORY T CELLS DO NOT DIRECTLY MODULATE ANTIGEN PRESENTING CELL RESPONSES TO MALARIA ANTIGEN IN A FILARIA/ MALARIA CO-ENDEMIC REGION

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We have previously shown that patent filarial infection is associated with an expansion of regulatory T cells (both adaptive [aTreg] and natural [nTreg]) and the IL-10 mediated suppression of malaria-induced production of IL-12, CXCL-9 (MIG), CXCL-10 (IP-10) and IFN-γ. Because nTregs have been shown to inhibit antigen presenting cell (APC) function by altering their maturation and modulating the expression of co-stimulatory molecules, we sought to determine whether the filarial-induced expansion of nTregs contribute to the diminished APC cytokine production following malaria antigen (MalAg) stimulation in a filarial/malarial co-endemic population of Mali. From filarial-infected (Fil+; n=18) or -uninfected (Fil-; n=19) individuals, purified nTregs (CD4+CD25+CD127low) and effector T (Teff) (CD4+CD25-CD127low) cells were cultured with purified APCs in the presence or absence of MalAg, and APC-derived cytokines measured. APCs from Fil+ individuals produced significantly lower levels of CXCL-9 ($p = 0.0006$) and IL-12 ($p = 0.02$) compared to APC from Fil- subjects. Although MalAg induced CXCL-9 ($p = 0.0009$), and CXCL-10 ($p = 0.0009$) as well as IFN-γ ($p = 0.0011$) in the APC:Teff co-culture of all subjects, the addition of nTreg to APC cultures did not directly alter the APC cytokine response to MalAg ($p > 0.05$) in either group. Surface marker blockade, previously implicated in the delivery of negative signals by nTregs (e.g GITR, PD1, LAG-3, IL-10R and CTLA-4) to APC failed to alter cytokine production when nTregs were added to APCs. In contrast, blocking PD1 or IL-10R in APC:Teff cocultures markedly augmented the production of IFN-γ, IL-12p70, CXCL-9 and CXCL-10 ($P < 0.05$ for all comparisons) in Fil+ subjects. This augmented cytokine production was significantly higher in Fil+ subjects compared to Fil- subjects. Our data clearly show that nTregs do not directly inhibit APC cytokine production in response to MalAg stimulation and suggest that expansion of nTregs is not responsible for the diminished cytokine production by APCs in filarial infections.

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HISTAMINE RELEASE DURING *LITOMOSIDES SIGMONDONTIS* INFECTION ENHANCES ADULT WORM BURDEN

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Numerous studies have demonstrated that helminth antigens induce release of histamine from basophils and mast cells of infected hosts. To date, however, the role histamine plays in the immune response against helminths has not been well characterized. In this study, we evaluated the role of histamine in mice infected with *Litomosoides sigmondontis*, a tissue-invasive filarial infection of rodents that lives for months in immunocompetent Balb/c mice. Extended time-course studies revealed that histamine in plasma peaked at 8 weeks of infection (mean 30nM at 4 wks, 325nM at 8 wks, and 100nM at 12 wks compared to 20nM in uninfected age-matched controls) whereas expression of histidine decarboxylase mRNA in circulating blood cells increased throughout the course of infection (fold increase over uninfected age-matched controls = 20 at 8 wks and 35 at 12 wks). Mice vaccinated with irradiated L3 larvae demonstrated substantial increases in circulating histamine levels 30 minutes after challenge infection (mean 400nM vs 20nM in unchallenged,

p<0.001), but administration of HR1 and HR2 receptor blockers did not attenuate the protective efficacy of vaccination (82% protection in untreated groups, 82% protection in HR1 treated, 80% protection in HR2 treated). Interestingly, short time course measurements demonstrated that primary infection of unvaccinated mice with L3s also causes histamine release into the bloodstream 30 minutes following infection (mean 200nm vs 20nM in uninfected, p<0.05), indicating a non-specific mechanism of histamine release. To evaluate the role histamine may play during infection, mice were chronically administered HR1, HR2, and a combination of HR1 and HR2 blockers in their drinking water and assessed for adult worm survival after inoculation with 40 L3 larvae. Surprisingly, at 8 weeks post-infection all groups of mice treated with antihistamine antagonists had significantly reduced numbers of adult worms compared to untreated controls (mean number of adult worms = 5 in HR1 group, 12 in HR2 group, 6 in HR1/HR2 group, and 20 in untreated infected control group, p values of <0.05 when comparing each treatment group against infected controls). Taken together, these data indicate that histamine, rather than being involved in vaccine-mediated protection, may be induced by filarial parasites for their growth and/or survival *in vivo*.

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MICROFILARIAE OF *BRUGIA MALAYI* INDUCE BOTH AN ALTERNATIVELY ACTIVATED AND PROINFLAMMATORY PHENOTYPE IN HUMAN MONOCYTES

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Monocyte dysfunction has been proposed as a cause of the reduced parasite-antigen specific T-cell response seen in patients with chronic filarial infections. Monocytes from these infected individuals internalize filarial antigens and express markers associated with alternatively activated macrophages (M). To understand the role of filarial antigens in monocyte differentiation, human monocytes from healthy volunteers were exposed to either live microfilariae (mf) of *Brugia malayi* or cytokines known to produce classically activated (combination of LPS and IFN-γ or MCSF) or alternatively activated (IL-4) phenotypes. The cells were then assessed for their expression of markers associated with alternative activation and their ability to phagocytose. Our data indicates that, similar to IL-4, mf significantly (p<0.05) upregulated mRNA expression of CCL15, CCL17, CCL18, CCL22, PDL1, and PDL2 but not Arg-1 in monocytes. Secreted products from mf or IL-4 significantly downregulated the monocyte mRNA expression of TLR3 and TLR7 resulting in decreased production of IL-6 following TLR ligand stimulation. Unlike IL-4, but similar to LPS/IFN-γ, mf significantly (p<0.05) upregulated the production of proinflammatory cytokines IL-1β, IL-6, neopterin, soluble ICAM-1, and TNF-α. Furthermore, both live mf and soluble factors from mf enhanced the cell surface expression of ICAM-1 on monocytes. Interestingly, mf significantly upregulated (4 fold) the mRNA expression of Indoleamine2, 3-dioxygenase (IDO) as well as its activity. Functionally both IL-4- and mf-exposed monocytes showed a significant decrease in their phagocytic ability compared to MCSF-cultured cells. Our data suggest that exposure of human monocytes to live mf of *Brugia malayi* induce monocytes to have characteristics of both alternative and classical ('proflammatory') activation. Further studies directed toward defining, at the single cell level, the multifunctionality of a given monocyte subset induced by filarial worms are ongoing.

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ONCHOCERCA VOLVULUS RECOMBINANT ANTIGENS OV-103 AND OV-RAL-2 ARE ASSOCIATED WITH PROTECTIVE IMMUNITY IN HUMANS AND INDUCE RESISTANCE TO INFECTION IN MICE

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Onchocerca volvulus (*Ov*) remains an important cause of blindness and chronic disability. While mass drug administration with ivermectin is ongoing, evidence is building for the existence of resistance to the drug. The development of an anti-larval vaccine would reduce adult worm burdens and thereby diminish microfilariae numbers in the skin resulting in reduced pathology and the interruption of transmission. Two *O. volvulus* vaccine candidates, Ov-103 and Ov-RAL-2 were chosen for study based in part on their homologues being highly protective in other nematode animal models. The antigens are expressed by the larvae in the basal layer of the cuticle and hypodermis. Ov-103 is also present in the basal lamina, channels connecting the esophagus to the cuticle and multivesicular bodies within the hypodermis. Ov-103 and Ov-RAL-2 are highly antigenic

in *Ov* exposed and infected populations with significant correlations between the IgG1 and/or IgG3 cytophilic antibodies responses and the development of protective immunity. The anti-Ov-103 IgG3 responses in the putatively immune individuals (PI) and the infected (INF) were elevated and similar while the anti-IgG1 responses were significantly higher in the INF. The anti-Ov-RAL-2 IgG3 responses in the INF are significantly increased with age, while the IgG1 is highly elevated regardless of age. To test the antigens' potential as vaccine candidates, mice were immunized with the antigens in alum. Yeast derived Ov-103 induced significant protection (31%) against larval *Ov*, while antigen from *E. coli* did not. Immunization with *E. coli* derived Ov-RAL-2 induced significant protection (44%), whereas the yeast produced antigen did not. These results suggest that the expression system has an effect of the potency on the recombinant vaccine antigens. We conclude that Ov-103 and Ov-RAL-2 are associated with protective immunity in humans and that both antigens induced significant levels of protective immunity in mice, thus making them potential candidates for a prophylactic vaccine against onchocerciasis.

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PROTECTIVE RESPONSE OF HOST NON-HOMOLOGOUS CONFORMATIONAL EPITOPES OF *WUCHERERIA BANCROFTI* THIOREDOXIN (*WB*-TRX)

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Vaccine studies in parasitic diseases have been a long term struggle due to the complex life cycles and immune evasion mechanisms. The present study explores the avenues of structural analysis on parasitic antigens to qualitatively improve protective immune response in mammalian hosts against nematode parasitic infections. In this regard, *Wuchereria bancrofti* thioredoxin (*Wb*-TRX) which protects filarial worms from radical-mediated damage of the host was selected. Accordingly, recombinant *Wb*-TRX was purified without fusion tag for structural studies. The enzyme activity was demonstrated using insulin reduction assay. The structure of *Wb*-TRX was solved by X-ray crystallography and was utilized for conformational epitope analysis. Since *Wb*-TRX shares sequence homology (~40%) with mammalian proteins, certain putative host-non-homologous regions from the protein sequence was selected and analysed by *in silico* immunoinformatic tools. These selected regions were located and analysed in the protein structure of *Wb*-TRX. Additionally, these regions were also validated against online conformational epitope databases. Based on these analyses, putative Discontinuous Epitope Peptide regions (DEP) were selected and synthesized. The activity assay performed for *Wb*-TRX in the presence of anti-sera raised in mice against these Discontinuous Epitope Peptides (DEP) characteristically reduced activity proposing a mechanism of enzyme inhibition depriving antioxidant ambience and challenging parasite survival. Further, a linear Peptide Conjugate (PC1) derivative of the DEP was evaluated for vaccine efficacy in permissive *Mastomys coucha* model. Interestingly, PC1 showed 75% greater protection compared to 63% protection of r*Wb*-TRX. Hence, the current study reports the utilization of conformational epitope enrichment through structural validation as a novel strategy for enhancing the efficacy of parasite antigens in host protection.

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FATTY ACID AND RETINOL-BINDING (FAR) PROTEINS OF FILARIAL NEMATODES: POTENTIAL VACCINE CANDIDATES FOR ONCHOCERCIASIS AND LYMPHATIC FILARIASIS

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The FAR proteins of filarial nematodes are helix-rich, fatty acid and retinol-binding (FAR) proteins that appear to be specific to nematodes and secreted into the surrounding tissues of the host. These proteins may play an important role in scavenging fatty acids and retinoids from the host and are probably essential for the survival of filarial nematodes and hence are considered as potential vaccine/drug targets. In previous studies, immunization with a fragment of the *Onchocerca volvulus* retinol-binding protein (Ov-RBD-1/Ov-FAR-1) induced significant resistance to challenge infection with third-stage larvae. Ov-FAR-2 was cloned by using serum from individuals who have developed concomitant immunity and Bm-FAR-2 was identified by bioinformatics. To further the development of recombinant vaccines against *O. volvulus* and lymphatic filariae, we focused on two members of the filarial FAR protein family. Recombinant FAR-1 and FAR-2 proteins from *O. volvulus* and *Brugia malayi* were expressed in *Escherichia coli* and *Pichia pastoris* and their ligand binding properties were compared using a fluorescence-based assay. The O. volvulus and *B. malayi* FAR-1 proteins bind to both retinol and DAUDA and can be displaced from DAUDA by structurally-related oleic acid. Surprisingly, both filarial FAR-2 proteins bind only to retinol and not to DAUDA, which suggests that FAR-2 and FAR-1 are different in structure and potentially also in function. The recombinant FAR proteins were tested for their efficacy as vaccine in the *O. volvulus* - mouse chamber model and in the *B. malayi* - jird animal model. Mice immunized with Ov-FAR-1 in alum, expressed in either *E. coli* or *P. pastoris*, killed 54% of the *O. volvulus* challenge infection. Immunization of jirds with recombinant Bm-FAR-1 expressed in E. coli and formulated with alum resulted in 41% reduction in worm count and immunization with Bm-FAR-1 formulated with the Montanide-720 adjuvant resulted in 74% reduction in worm count. Further vaccination experiments investigating the FAR-2 molecules in these two animal models are underway. Correlation between the immune responses in humans to the FAR proteins vs. those in the protected mice and jirds will be presented. In conclusion, the FAR proteins of filarial nematodes are excellent candidates for use in prophylactic vaccines against infection with *O. volvulus* and *B. malayi*.

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FILARIAL LYMPHATIC PATHOLOGY REFLECTS ELEVATED LEVELS OF CIRCULATING INFLAMMATORY BIOMARKERS OF LYMPHATIC AND IMMUNE DYSFUNCTION

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Infection with *Wuchereria bancrofti* can be associated with development of serious pathology in the form of lymphedema, hydrocele, and elephantiasis in a subset of infected patients. Dysregulated host inflammatory responses, lymphatic dysfunction, endothelial activation and extracellular matrix remodeling play central roles in filarial disease pathogenesis. To identify factors contributing to pathogenesis of disease in lymphatic filariasis, we examined the role of microbial translocation markers (LPS, LBP, EndoCAb and sCD14); acute phase proteins [α-2

Macroglobulin (α-2 m), Haptoglobulin, C-reactive proteins (CRP) and Serum Amyloid protein-A (SAA)]; angiogenic factors (VEGF - A, C, D, R1, R2 and R3 and Angiopoietin -1 and 2); pro- and/or anti-fibrotic factors (MMP - 1, 7, 8 and 9 and TIMP - 1, 2, 3 and 4) and pro-inflammatory cytokines (IFNγ, TNFα, IL-12, IL-1β, IL-6, IL-17 and GM-CSF) in chronic filarial pathology with (CP Ag+ (n=24)) or without (CP Ag- (n=65)) active infection as well as in asymptomatic, infected (INF; n=84); and uninfected, endemic normal (EN; n=64) individuals. Markers that were significantly elevated in CP Ag+ compared to INF but not in CP Ag- compared to EN individuals were considered to truly reflect biomarkers of pathogenesis. CPAg+ individuals had significantly elevated plasma levels of LPS (p=0.0001), α-2m (p=0.0003), haptoglobulin (p<0.0001) and SAA (p=0.0385) among the microbial translocation and acute phase panels. Among the angiogenic growth and fibrotic factors, we found significantly elevated levels of VEGF-A (p=0.0031) and C (p<0.0001), VEGF-R1 (p=0.0033), R2 (p<0.0001), R3 (p=0005) and Angiopoietin-1 (p=0.0481) but not the MMP/TIMP family. In addition, a variety of pro-inflammatory cytokines including IFNγ, IL-12, GM-CSF (p<0.0001 for all) and IL-1β (p=0.0073) were significantly elevated in CP Ag+ individuals. The elevated levels of these factors suggest quite strongly that the alteration of lymphatic integrity and peri-lymphatic inflammation should be implicated in the pathogenesis of lymphatic filarial pathology.

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DOES MALARIA IN PREGNANCY IMPAIR PLACENTAL DEVELOPMENT? EVIDENCE FROM AN *IN VITRO* MODEL

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In malaria endemic areas, *Plasmodium falciparum* (Pf) malaria during pregnancy is the leading preventable cause of low birth weight and neonatal mortality, often due to fetal growth restriction (FGR). The underlying pathogenic mechanisms are poorly characterized, but may include impaired placental development. The peak prevalence of maternal Pf infections between 13-18 weeks gestation coincides with the establishment of the placental circulation, when extravillous trophoblasts (EVT) invade the maternal uterus and transform maternal spiral arteries increasing placental blood supply. Adequate trophoblast invasion is essential for the establishment of appropriate placental function and successful fetal growth and impairment of this process is associated with other causes of human FGR. To address the impact of malaria infection early in pregnancy on placental development, we tested serum from Papua New Guinean women with Pf in peripheral blood at their first antenatal presentation (between 16 and 22 weeks gestation) for the ability to inhibit first trimester EVT-cell line invasion and viability *in vitro*. Compared to uninfected controls, serum from malaria-infected women significantly reduced trophoblast invasion (P <0.001). This phenomenon could not be explained by changes in trophoblast viability (P =.2). Because trophoblast invasion is enhanced by a number of hormones, cytokines and chemokines, and is inhibited by pro-inflammatory cytokines, many of which are dysregulated in malaria in pregnancy, we further compared concentrations of known modulators of trophoblast invasion in maternal blood between the groups. Serum collected from malaria-infected women had significantly lower levels of trophoblast invasion promoting factors (Insulin like growth factors -1 and -2, P =.0001, P =.01 respectively, and IL-8 P = 0.02) and higher levels of invasion inhibitory modulators (human chorionic gonadotrophin P =.002, IL-10 P =.01). Although malaria-induced elevated pro-inflammatory cytokines and reduced fetal growth hormones have been reported at delivery, this study is the first to describe altered levels of such factors early in pregnancy. These inflammatory and hormonal disturbances in early pregnancy may impair placental development. This is a significant advancement in our understanding of the temporal and pathophysiological events that may contribute to FGR due to Pf malaria in pregnancy.

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ANTENATAL CLINIC ATTENDANCE, INTERMITTENT PREVENTIVE TREATMENT USE AND PREGNANCY OUTCOME AMONG PARTURIENT WOMEN ATTENDING ADEOYO TEACHING HOSPITAL, IBADAN, OYO STATE, NIGERIA

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Intermittent Preventive Treatment in pregnancy with Sulfadoxine Pyrimethamine (IPTp-SP) is currently the recommended method for control of malaria in pregnancy. However, in Nigeria the proportion of women who take the recommended two doses during Antenatal Clinic (ANC) is low. In this study, we investigated the relationship between number of ANC visits and IPTp coverage, relationship between IPTp use and prevalence of malaria parasite at parturition in both mother and baby as well as pregnancy outcome.339 mother-baby pairs were enrolled at delivery in a secondary health care facility in Ibadan, south western Nigeria. An interviewer administered questionnaire was used to collect information on demographic details, history of index pregnancy. Thick blood films from maternal finger prick and neonatal heel prick were prepared and stained with fresh Geimsa stain. Data was summarized using frequency tables and means while differences in proportion were compared using Chi-square test. There were six twin delivery giving 339 mothers and 345 neonates. Mean age of mother was 28.1 ± 0.281 years, 126/339 (37.2%) mothers were primigravida, 80/339 (23.6%) were secundigravidae, 133/339 (39.2%) had had 3 pregnancies and above. Use of IPTp-SP was reported by 88/339 (26%) parturient women and 17/88 (19.3 %) of them received two doses before delivery. Coverage of IPTp-SP was significantly higher among women who had ≥4 ANC visits (32.2%) as compared with women who <4 visits (15.6%). There was no significant relationship between number of ANC visits and transportation cost as well as distance of residence to clinic. The prevalence of malaria parasite in the parturient women and neonates were 13.3% and 3.5% (12/345). Prevalence of malaria parasitemia was lower (0%) in those who used 2 or more doses of SP than in those who used less than 2 doses 4/71 (5.6%). There was no significant relationship between the number of doses of SP taken and maternal and neonatal haematocrit, birth weight and gestational age at parturition. Compliance with IPTp-SP use is ensured by frequent ANC visits and reduces level of parasiteamia in both mother and child. Effort at malaria control should be targeted more at encouraging women to attend antenatal clinic regularly to achieve completion of recommended dose of intermittent preventive treatment.

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PARASITOLOGIC ASSESSMENT OF TWO-DOSE AND MONTHLY INTERMITTENT PREVENTIVE TREATMENT OF MALARIA DURING PREGNANCY WITH INTERMITTENT PREVENTIVE TREATMENT WITH SULPHADOXINE-PYRIMETHAMINE (IPTP-SP) IN LAGOS, NIGERIA

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Intermittent preventive treatment of malaria with sulphadoxine-pyrimethamine (IPTp-SP) is a key strategy in the control of malaria in pregnancy from the second trimester. However, there is no data on the protective efficacy of IPTp-SP in Lagos, Nigeria. High sulphadoxine-pyrimethamine (SP) resistance reported among *Plasmodium falciparum*

isolates has been reported from clinical trials and molecular studies in children. This has necessitated the continuous monitoring of the efficacy of SP in pregnant women. Reports of malaria prevalence in Nigeria suggest that malaria is hyperendemic in most areas, thus raising concerns on the adequacy of the standard two-dose IPTp-SP strategy adopted for HIV-negative pregnant women. This study was done to determine the protective efficacy of IPTp-SP; and to assess the equivalence of monthly-dose to the standard two-dose IPTp-SP in Lagos. The study was a longitudinal study. The women were randomly allotted to two arms: two-dose IPTp-SP (Arm A) and monthly dose IPTp-SP (Arm B). A total of 259 pregnant women [Arm A=122; Arm B=137] attending antenatal clinics in two hospitals in Lagos, Nigeria were recruited. Eligibility criteria were the absence of symptomatic malaria, HIV and multiple pregnancy. Outcome measures were: absence of malaria parasites in peripheral blood; proportion of live births and low birth weight. Baseline parasitaemia (M0) in the two group was 5(4.1%) and 3(2.2%) in Arms A and B respectively. The overall protective efficacy of IPTp-SP was 98.4% (Arm A, 98.3% and Arm B, 98.5%) at M1(P=0.636). Similar result was obtained at the second month (M2) (P = 0.466). However, none of the women in the monthly IPTp-SP (Arm B), developed parasitaemia after M1; while a woman became parasitaemic at M2 in the 2-Dose IPTp-SP group (Arm A). The monthly dosing was not superior to the two-dose regime. The proportion of live births and low birthweight were similar in the two study arms (P>0.05). Intermittent preventive treatment of malaria during pregnancy with SP is effective in protecting pregnant women from malaria infection in Lagos. Monthly-dose IPTp-SP is equivalent to the standard two-dose IPTp-SP in Lagos, Nigeria.

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ACQUISITION AND MAINTENANCE OF IGG RESPONSES TO *PLASMODIUM FALCIPARUM* AND *P. VIVAX* DURING PREGNANCY

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Pregnant women are more susceptible to, and more severely affected by, malaria and other infectious diseases. In malaria endemic regions pregnant women typically develop high parasite densities, placental infection and associated complications, despite substantial immunity to malaria that may have been acquired prior to pregnancy. This has largely been attributed to both the modulation of maternal immune responses and the sequestration of *Plasmodium falciparum* parasites in the placenta. However, data on the acquisition and maintenance of antibody responses throughout pregnancy, and their relation to malaria is unclear. Furthermore, there are limited data on malarial immunity among pregnant women in low transmission settings, in Asia, and in a setting where *P. falciparum* and *P. vivax* are prevalent. In a nested case-control study of pregnant women on the Thai-Burmese border, we measured IgG levels to *P. falciparum* merozoite antigens (AMA1, EBA-175, MSP2, MSP3, schizont extract) and *P. vivax* merozoite antigens (Pv-AMA1) and the *P. falciparum* pregnancy-specific antigen VAR2CSA-DBL5 at 2-weekly intervals during pregnancy until delivery in 136 malaria cases and 124 controls (over 2000 samples in total). ELISAs were performed using novel high-throughput technology to facilitate determination of antibody levels in a large number of samples. Longitudinal analysis revealed that at the individual level, antibody responses could be grouped as dynamic or relatively stable during gestation. The most dynamic species-specific responses were seen in women who experienced active infection during pregnancy and the biggest magnitude of effect seen with VAR2CSA-DBL5 compared to merozoite responses. At the population level, antibody titres increased with gestation time in those with concurrent *P. falciparum* parasitaemia most likely reflecting boosting of responses with each successive infection. This study provides the most comprehensive analysis, to date, of antibody dynamics towards two *Plasmodium* spp. and contributes to our understanding of malaria during pregnancy and immune responses to infectious diseases during pregnancy.

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MALARIA IN EARLY PREGNANCY ADVERSELY AFFECTS *IN UTERO* FETAL GROWTH

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Maternal malaria produces adverse maternal and fetal outcomes, including maternal anemia and intrauterine growth restriction (IUGR). There is a scarcity of research regarding the effects of early malaria on pregnancy outcomes despite, typically, a lack of prevention and control measures during this critical time of placental development. We evaluated the effect of malaria parasitemia prior to 21 weeks' gestation on estimated fetal weight (EFW) and IUGR among a sample of 128 pregnant women enrolled prior to 21 weeks' gestation in a longitudinal ultrasound study from May 2005 to May 2006 in Kinshasa, DR Congo. Malaria exposure and ultrasound estimated fetal weight were measured monthly until delivery. Intermittent preventive treatment in pregnancy (IPTp) was provided twice (from 16-27 and 28-32 weeks' gestation), insecticide treated nets were provided, and slide-positive malaria cases were treated. Linear mixed models were fitted to estimate beta coefficients and 95% confidence intervals (CIs) to describe the effect of early malaria parasitemia on the mean difference in subsequent EFW; log-binomial general estimating equation (GEE) regression models were fitted to estimate risk ratios (RRs) and 95%CIs for effect of early malaria on risk of subsequent IUGR. Twenty-one percent of pregnant women had malaria parasitemia prior to 21 weeks' gestation and 43% ever experienced an IUGR episode after 21 weeks' gestation. Primigravidae with early malaria parasitemia had an approximately 80 gram decrease in EFW compared to primigravidae with no early malaria (95%CI: -116, -34). Primigravidae with early malaria had 3.6 times the risk of subsequent IUGR compared to the referent group of multigravidae with no early malaria parasitemia (95%CI: 2.1, 6.2). Early malaria was also associated with a small, non-significant increased risk of IUGR among multigravidae (RR: 1.4; 95%CI: 0.8, 2.5). Our findings indicate that appropriate malaria prevention and control efforts should begin earlier in pregnancy in order to prevent the adverse effects of malaria on fetal growth.

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EVALUATION OF SULFADOXINE-PYRIMETHAMINE FOR INTERMITTENT PREVENTIVE TREATMENT OF MALARIA IN PREGNANCY--- MANSA, ZAMBIA, 2010

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Intermittent preventive treatment of malaria in pregnancy (IPTp) with sulfadoxine-pyrimethamine (SP) decreases placental parasitemia and maternal anemia, thus improving birth outcomes, especially among primigravidae. Zambian policy recommends three SP doses, given presumptively to pregnant women, spaced one month apart after 16 weeks of gestation. Given increased parasite resistance to SP, we evaluated the effectiveness of SP for IPTp. HIV-negative pregnant women were enrolled during delivery at two facilities in an area of high malaria transmission. Women were interviewed, SP exposure was determined from antenatal records and self-report, and blood and placental samples were taken. We evaluated outcomes of maternal parasitemia, maternal anemia (Hb<11 g/dl), and placental parasitemia in women who took ≥2 (n=286) versus <2 (n=149) SP doses. The median age of participants was 23 years (range 16-44). Among all women, ≥2 SP doses provided no protection from maternal parasitemia [n=435, unadjusted odds ratio (OR) 1.0, (95% confidence interval (CI) 0.5-2.0)], anemia (OR 0.7, 95% CI 0.5-1.1), placental parasitemia (OR 0.9, 95% CI 0.4-2.0), and placental monocyte infiltration, (OR 0.9, 95% CI 0.4-2.0). We found similar, non-significant results when evaluating ≥3 SP doses versus <2 doses. However, taking ≥2 SP doses was protective against anemia among primigravidae (n=159, OR 0.4, 95% CI 0.2-0.9). Maternal age, urban living, and use of insecticide-treated nets were not significantly associated with outcomes. Controlling for age, vaginal delivery, marital status, and living in a house with indoor residual spraying, taking ≥2 SP doses remained protective for anemia among primigravidae (OR 0.4, 95% CI 0.2-0.7). SP for IPTp may confer less protection against malaria than previously observed. Limitations of this study include strata too small to analyze [e.g. severe anemia (Hb<8 g/dl), and no SP taken], insufficient power to examine birth weight as an outcome, and unverified SP quality. These findings emphasize the urgent need to evaluate alternative medications for IPTp.

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LINKING MALARIA IN PREGNANCY TO POPULATION TRANSMISSION DYNAMICS: A MODEL OF THE PROGRESSION OF PLACENTAL INFECTION AND THE ROLE OF PARITY-DEPENDENT IMMUNITY

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Malaria in pregnancy, which is associated with maternal anaemia, preterm birth, low birthweight and increased mortality for both mother and child, continues to be a major public health issue in many parts of the world. Ensuring that prospective mothers are provided with the most effective treatment or preventative measures is a priority. However, assessment of the effectiveness of any intervention is problematic because the infection status of the placenta can only be assessed at delivery. Thus, for the preceding period of gestation, the prevalence of malaria in pregnancy can only be estimated from peripheral blood which has been shown to be neither a sensitive nor a specific indicator of placental infection. To improve estimation of the prevalence of infection directly from placental prevalence, a mathematical model linking the progression of placental infection throughout pregnancy to an existing model of the prevalence of malaria within the general population was developed which includes both age and parity as factors which affect the dynamics of infection. The model was fitted to placental histology data (prevalence of acute, chronic and past infection by age and parity) from two transmission settings (Kilifi, Kenya, 1995/96, EIR≈5 and Ifakara, Tanzania, 1994/95, EIR≈365) using Bayesian MCMC methods to provide estimates of the rate of progression of infection within the placenta. Our results suggest that parity-specific immunity which results in faster clearance of placental infection is more consistent with patterns of placental infection in different parities than an immunity mechanism that decreases the probability that an acquired peripheral infection sequesters the placenta. Furthermore, we estimate that the duration of chronic infection decreases rapidly with exposure to infection in previous pregnancies with a single prior infection reducing this time by over two-thirds. From these parameters we derived the relationship between transmission intensity and age- and parity-specific prevalence of placental infection which can be used to aid the assessment of appropriate intervention strategies in areas of differing transmission intensity.

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LONGITUDINAL MOLECULAR EPIDEMIOLOGY OF PREGNANCY-ASSOCIATED MALARIA IN BLANTYRE, MALAWI

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Pregnancy-associated malaria (PAM) is an important cause of poor birth outcomes in sub-Saharan Africa, though its prevalence is decreasing. From 1997 to 2005, during which the prevalence of parasitemia at delivery decreased from 24% to 5% at a single hospital in Blantyre, Malawi, we analyzed trends in molecular markers of drug resistance and parasite diversity among 358 *Plasmodium falciparum* isolates from the peripheral blood of delivering women. The average multiplicity of infection (MOI), as determined by merozoite surface protein-2 (msp2) genotyping, decreased from 3.8 (standard deviation 1.3) to 1.8 (SD 1.2) during the study period. The prevalence of the triple-mutant dihydrofolate reductase (dhfr) haplotype increased from 33% to 100%, and that of the double-mutant dihydropteroate synthase (dhps) haplotype from 33% to 100%; concomitantly, the prevalence of the combined quintuple-mutant haplotype increased from 17% to 100%. In contrast, haplotypes of the *P. falciparum* multidrug resistance gene (pfmdr1) were unchanged over time, and mutations at codon 164 of dhfr and codon 581 of dhps failed to emerge. Accounting for parasite multiplicity, we calculated frequencies of alleles and haplotypes and used these to compute selection coefficients as a quantitative index of the strength of selection upon mutant alleles and haplotypes. The results demonstrate the molecular correlates of improved control of pregnancy-associated malaria, and serve as a model for the emergence and fixation of drug-resistance in a semi-immune population exposed to increasing sulfadoxine-pyrimethamine. Clinical correlation of these phenomena will inform drug therapy and resistance surveillance policies.

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GENOMIC DIVERSITY OF VAR2CSA DUFFY-BINDING-LIKE DOMAINS IN PREGNANCY-ASSOCIATED MALARIA EXPLORED WITH MASSIVELY-PARALLEL PYROSEQUENCING

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VAR2CSA expression on the surface of erythrocytes infected by *Plasmodium falciparum* mediates placental malaria by allowing for the sequestration of infected erythrocytes in the placenta. Because of this specific molecular pathogenesis, VAR2CSA has been advanced as a target for a vaccine against pregnancy-associated malaria. To do so, pathogenic and immunogenic motifs must be identified within var2csa, but these efforts have been bedeviled by the sequence diversity of var2csa. Genomic diversity between and within populations of parasites that comprise a single infection is not adequately described by traditional genotyping methods because they fail to capture all variants. To this end, we employed massively-parallel pyrosequencing to explore the diversity of two var2csa Duffy binding-like (DBL) domains in genomic DNA of parasites infecting the placentae and peripheral blood of delivering women in Malawi. After amplifying across hypervariable regions of DBLs 5 and 6, amplicons were sequenced on a Roche 454 System, which sequences individual amplicons up to 500bp long. We used population genetic analysis methods to compare populations of parasites between women, between study sites, and between peripheral and placental blood, and we computed DBL variant discovery curves. The combination of next-generation sequencing and population genetic analysis provides a method by which to capture and exploit the genomic diversity of *P. falciparum* within individual infections. These tools may aid the identification of motifs that are critical to the pathogenicity and immunogenicity of pregnancy-associated malaria.

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INTENSIVE SURVEILLANCE FOR MALARIA DURING PREGNANCY

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In studies of pregnancy-associated malaria, surveillance is frequently limited to examination of the placenta for evidence of active or past malaria infection. The sensitivity of placental histology for the detection of malaria during pregnancy is not well studied. We conducted a prospective observational study recruiting women in their first or second pregnancy who were less than 28 weeks gestation and attending the Ndirande Antenatal Clinic in Blantyre, Malawi for the first time. Participants had microscopy and hemoglobin measurement done monthly and whenever they were ill. All women received three doses of intermittent preventive therapy. At delivery, a maternal peripheral, placental and cord blood, and placental biopsy samples were collected. A total of 450 women were recruited; 63% (n=285) were primigravidae. Among the 342 women with evaluable birth outcomes, 38 (11%) had malaria at enrollment and an additional 22 (6%) had malaria detected on peripheral blood smear after enrollment. On histological evaluation, 62/342 (18%) had evidence of placental malaria based on the presence of parasites and/or pigment. Fifty six percent of women with positive malaria smears during pregnancy had evidence of malaria infection in the placenta and 43% without peripheral parasitemia had placental malaria. There was no association between timing of malaria infection and the likelihood of detecting placental malaria. This study did not have the power to detect the relationship between these measures of malaria exposure and low birth weight or maternal anemia. We will report the relationship between malaria and gestational age at delivery based on ultrasound results. The first half of pregnancy, before women typically seek out antenatal care, is a period of vulnerability to malaria infection. Placental histology identified approximately half of women who had detectable infection in the peripheral blood during pregnancy. Studies with large sample sizes are needed to determine if histology is an adequate surrogate for intensive surveillance in detecting the adverse outcomes associated with malaria during pregnancy.

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THERAPEUTIC EFFICACIES OF ARTEMISININ-BASED COMBINATION THERAPIES IN NIGERIAN CHILDREN WITH UNCOMPLICATED *FALCIPARUM* MALARIA DURING FIVE YEARS OF ADOPTION AS FIRST-LINE TREATMENTS

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The therapeutic efficacies of 3-day regimens of artesunate-amodiaquine and artemether-lumefantrine, during 5 years of adoption as first-line treatments, were evaluated in 811 <12 year-old malarious children. Compared with artemether-lumefantrine, amodiaquine-artesunate significantly reduced the proportion of children with fever and parasitemia 1d after treatment began (day 1) (P <0.008 for both). The proportion of parasitemic children on day 2, and gametocytemia on presentation and carriage reduced significantly over the years (P <0.000001, and P <0.03, respectively, test for trend). Overall efficacy was 96.5% (95%CI 94.5-98.6) and remained unchanged over the years (P = 0.87, test for trend). Kinetics of parasitemias following treatments was estimated by non-compartmental model. Declines of parasitemias were monoexponential with mean elimination half-life of 1.09h (95%CI 1.0-1.16). Parasitemia half lives and efficacy were similar for both regimens and in all ages. Artesunate-amodiaquine and artemether-lumefantrine remain efficacious treatments of uncomplicated *falciparum* malaria in Nigerian children 5 years after adoption.

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EVALUATION OF ADVERSE DRUG REACTIONS TO ARTEMISININ-BASED COMBINATION THERAPY IN A COMMUNITY HEALTH CLINIC IN PAPUA PROVINCE, INDONESIA

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Malaria is one of the main health problem in east of Indonesia. Since 2004, Ministry of Health Republic of Indonesia has recommended the use of orally administered Artemisinin-Based Combination Therapy (ACT) to treat uncomplicated malaria. However, some patients experienced uncomfortable adverse reactions due to ACT, leading to its discontinuation. Recognition of these adverse reactions may lead to better management. The aim of this study is to determine the prevalence of adverse reactions due to orally administered ACT and types of reactions experienced by uncomplicated malaria patients. A cross sectional study among uncomplicated malaria patients was conducted in Community Health Clinic Medical Emergency and Rescue Committe, a non-governmental health organization, in Timika, Papua, Indonesia, between September 2010 and March 2011. Sample was collected consecutively. Primary data were collected by interview using a questionnaire to assess

adverse reactions experienced by the patients, while demographic data were obtained from medical records. There were 250 uncomplicated malaria patients who fulfilled the inclusion criteria and did not meet any of the exclusion criteria. Adverse reactions of orally administered ACT were experienced by 48% respondents. Most common adverse reaction was nausea (28%). Approximately 33% respondents who experienced adverse reactions considered them intolerable. These findings are much higher compared to other studies. Adisa et al (2008) and Cairo et al (2008) found adverse reactions due to ACT in 32.9% and 8.8% respondents respectively. Nausea, the most common adverse reactions found in this study, was found only in 1,3% patients interviewed by Adisa et al and 16% of Cairo et al's subjects. This difference is probably due to different types of ACT used and dosage given to the patients. Eventhough, only 33% respondents who experienced adverse reactions considered these adverse reactions intolerable, around 22% of these respondents discontinued their ACT against medical advice. In conclusion, prevalence of adverse reactions due to orally administered ACT was quite frequent. Efforts should be made to minimize the percentage of patients discontinuing their medication. Patients should understand the adverse reactions of ACT that might occur and doctors should carefully manage these adverse reactions.

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POPULATION PHARMACOKINETICS OF ARTESUNATE AND DIHYDROARTEMISININ IN HEALTHY AND MALARIA-INFECTED SUBJECTS

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Pyramax® is a pyronaridine/artesunate combination currently under evaluation for treatmentof uncomplicated malaria in adult and pediatric patients. We analyzed the population pharmacokinetics of artesunate (AS) and its active metabolite dihydroartemisinin (DHA) in healthy and malaria-infected subjects participating in eight Pyramax® clinical trials. Pharmacokinetic data were available from 166 healthy Caucasian and Korean adults and 631 African and Asian adult and pediatric patients with mild to moderate uncomplicated malaria. Plasma AS and DHA concentrations were measured using a validated LC-MS method. Non-linear mixed effects modeling was used to obtain the pharmacokinetic and variability parameter estimates. A simultaneous parent-metabolite model was developed consisting of first-order absorption of AS, a one-compartment model for AS, and a one-compartment model for DHA. The population estimates showed that AS absorption was rapid, with an absorption rate constant (Ka) of 3.02 h^{-1}. Apparent AS clearance (CL/F) and volume of distribution (V2/F) were 1170 L/h and 1040 L, respectively. Estimates obtained for apparent clearance (CLM/F) and volume of distribution (V3/F) of DHA were 82.0 L/h and 107 L, respectively. Stepwise covariate modeling yielded four significant covariate-parameter relationships: weight on CL/F, CLM/F, and V3/F and ritonavir coadministration on CLM/F. Ritonavir coadministration was associated with a substantial increase in CLM/F of 30.5 L/hr. Malaria infection did not exert a significant influence on any pharmacokinetic parameter. Creatinine clearance, hepatic enzyme elevations, and gender also did not meet the model inclusion criteria. Estimated inter-individual variability was greatest for Ka (159%), followed by V2/F (34.6%). Evaluation of the final model using bootstrapping, visual predictive check, and condition number indicated that the model displayed satisfactory robustness, predictive power, and stability. The final model was utilized to simulate AS and DHA concentration-time profiles for representative weights in each of four Pyramax® dosing groups.

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PHARMACOKINETICS, SAFETY AND EFFICACY OF ARTEMISININ-NAPHTHOQUINE COMBINATION (ARCO™) THERAPY IN PAPUA NEW GUINEAN CHILDREN WITH UNCOMPLICATED *PLASMODIUM FALCIPARUM* AND *P. VIVAX* MALARIA

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Artemisinin-naphthoquine (AN) is available as a fixed-dose oral co-formulation marketed in Papua New Guinea (PNG) and other countries as ARCO™ (Kunming Pharmaceutical Company). It is currently recommended as single-dose treatment even though the WHO stipulates that artemisinin-based combination therapy should be given as a 3-day regimen. To date there have been limited published data on the pharmacokinetics of naphthoquine, particularly in children. We conducted a randomised study in PNG children aged 5-12 years with uncomplicated *falciparum* or *vivax* malaria comparing single-dose ARCO (A:N 15:6 mg/kg) given with water (Group A, n=15), single-dose (A:N 22:9 mg/kg) administered with full-cream milk (Group B, n=17) or as two daily doses (A:N 22:9 mg/kg) given with water (Group C, n=16). Blood samples were collected from each child at 15 time points after dose, and naphthoquine in plasma was quantified by liquid chromatography-mass spectrometric assay. Of the 48 children (46 with *falciparum* and 2 with *vivax* malaria), 2 in Group B withdrew because of inability to complete study procedures. All regimens were well tolerated with no serious adverse events. There were no significant changes in pulse, blood pressure, rate-corrected QT interval, or routine biochemistry after treatment, and fever clearance was prompt. The mean 50% parasite clearance times were 3.9, 3.9 and 4.6 h for Groups A, B and C, respectively. In Group A, 1 patient had Day 23 parasitological failure, and 2 children presented with re-infections on each of Days 28 and 42. In Group B and C there were no cases of parasitological failure but 1 Group B child had a re-infection on Day 42. Gametocyte clearance was prolonged with 20%, 27% and 9% of participants still positive on Day 14 in Groups A, B and C, respectively. Using a two-compartment model, the mean terminal elimination half-lives of naphthoquine were 420, 372 and 453 h for Group A, B and C, respectively. The maximum plasma concentration after dose was similar for all three groups. Both single and two-dose ARCO regimens were safe and well tolerated. The long half-life of naphthoquine is similar to those reported for 4-aminoquinolines and related compounds and protects against recrudescence. Milk co-administration did not appear to influence naphthoquine pharmacokinetics.

1497

IN VITRO "PITTING" TO DETECT ARTEMISININ-RESISTANT *PLASMODIUM FALCIPARUM*?

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Artemisinin-based therapies are currently the reference treatment for malaria worldwide. *Plasmodium falciparum* resistant to artemisinin derivatives is spreading in South-East Asia. Tools that would rapidly detect the onset of resistance are urgently needed. Conventional assessment of *P. falciparum* drug resistance *in vitro* is based on the determination of the IC_{50}, but IC_{50} of artesunate or dihydroartemisinin (DHA) only weakly correlate with parasite clearance in artemisinin-treated patients. In these patients, *P. falciparum* parasites are expulsed from their host red blood cell by the spleen, a "pitting" process that greatly accelerates parasite clearance, as reported previously. We want to use *in vitro* pitting as a test to assess parasite resistance in a new, physiologically relevant way. *In vitro*

pitting will indeed mimic the major natural parasite clearance mechanism. Using an ex-vivo human spleen model, we had obtained the pitting of red blood cells containing artemisinin-induced parasite remnants, as reported previously. We had also observed that parasite remnants are deposited along the wall of red pulp sinuses in the spleen of an artemisinin-treated malaria patient. We recently established and validated an *in vitro* red blood cell filtering device that mimics the mechanical sensing of red blood cells as they cross the wall of red pulp sinuses in the human spleen, as reported previously. Using this device we have obtained the pitting of parasitized red blood cells previously exposed to artesunate or DHA. More than 25% of artesunate-exposed parasitized red blood cells are pitted by a single passage through the filters. This filtering process requires less than 50 µl of peripheral blood and less than 5 hours of parasite exposure to the drug. We are currently identifying the major determinants of the *in vitro* pitting process, and will soon determine the pitting rate of parasite isolates from patients with fast or slow parasite clearance upon treatment with artesunate.

1498

DECREASING *IN VITRO* SUSCEPTIBILITY OF FRESH *PLASMODIUM FALCIPARUM* ISOLATES TO DIHIDROARTEMISININ IN COLOMBIA

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The main drawback to controlling malaria is the emergence and spread of multidrug resistant parasites. Artemether+lumefantrine therapy was implemented during 2007 in Colombia for *falciparum*-malaria, showing excellent outcomes. Recent reports from Southeast Asia show that it is likely that resistance to Artemisinin Combined Therapies-ACTs is developing. This phenomenon highlights the need to keep monitoring systems for early detection of resistance in other endemic areas. Our aim was to determine the spatial/temporal changes in the *Plasmodium falciparum* phenotypes through the first years of ACTs implementation in Colombia. From 2008 to 2010, 121 isolates from Quibdó and Tumaco, (North and South Colombian Pacific coast) were evaluated for *in vitro* susceptibility to mefloquine-MQ, lumefantrine-LUM and dihidroartemisinin-DHA, through microscopic and ELISA-HRP2 tests. For quality control, the reference strain W2 was evaluated. The IC_{50}s were calculated using NH-nonlin software and non-parametric tests were used to compare the results. In 2010, DHA showed a significant increased IC_{50}s ($p<0.05$) in Tumaco (Geometric Mean-MG in 2008: 2.02nM, 2009: 1.2nMand 2010: 4.0nM). Meanwhile, during 2008-2010, LUM and MQ IC_{50}s were stable in both regions. The parasites from Quibdó were less susceptible to MQ (MG in 2008: 36nM, 2009: 47.8nMand 2010: 30.7nM) compared to those from Tumaco (MG in 2008: 14.3nM, 2009: 25.1nM and 2010: 15.8nM) in this period ($p<0.05$). Spearman correlation test between both methodologies was 0.92 ($p<0.01$). The fact that *in vitro* DHA IC_{50}s are increasing in Tumaco highlights the need of continuing with active surveillance. Although all the isolates tested showed a high susceptibility to DHA and LUM, which is comparable to the high efficacy observed in Colombia with ACTs. Because there is cross *in vitro* susceptibility between LUM and MQ, our findings underline the importance of close monitoring of the *in vitro* response to LUM in the North Pacific region. *In vivo* studies are costly and not sensitive for early detection of resistance (due to the high efficacy of ACTs) and there are no molecular resistance markers to artemisinin derivates. Therefore *in vitro* assays are currently the main strategy for early detection of resistance to ACTs.

1499

THE INFLUENCE OF NEVIRAPINE ON ARTESUNATE AND DIHYDROARTEMISININ EXPOSURE IN HIV-INFECTED NIGERIAN ADULTS

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Nevirapine (NVP)-based antiretroviral therapy (ART) may result in important drug-interactions with some antimalarials. Artesunate plus amodiaquine (AS-AQ) is a common antimalarial combination used in Nigeria and the pharmacokinetic (PK) effect of combining AS-AQ with NVP is unknown. AS and AQ are converted to active metabolites via hydrolysis and cytochrome p450 pathways, respectively; the latter being most susceptible to drug-drug interactions. We conducted a two-group comparison of AS-AQ PK in HIV-infected patients receiving NVP-based ART for at least 8 weeks (n=10) to HIV-infected patients not on ART (control; n=11). AS-AQ 200/600mg was given as a daily dose for three days. Blood sample collection for the measurement of AS and AQ was commenced following the final dose at 0 (pre-dose), 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24, 48, 72 and 96h. AS and the active metabolite dihydroartemisinin (DHA) were quantified with LC-MS/MS. PK parameters [maximum concentration (C_{max}; mg/L), (volume of distribution (Vd; L), area under the curve (AUC; mg/L/h), oral clearance (Cl; L/hr), and half-life ($_{t1/2}$; h)] were determined with non-compartmental analysis (mean ± standard deviation). In the NVP group compared to controls, the AS Vd was 1162 ± 856 vs 4525 ± 3535 ($p=0.01$) and Cl was 1950 ± 543 vs 2995 ± 1180 L/h ($p=0.03$), while the AUC was 105 ± 31 vs 69 ± 26 ($p=0.02$). AS $_{t½}$ was 0.4 ± 0.3 in the NVP group and 1.1 ± 0.9h in controls ($p=0.06$), while the AS C_{max} was 108 ± 42 vs 71 ± 57 ($P> 0.05$), respectively. DHA AUC (603 ± 218 vs 883 ± 607) and C_{max} (298 ± 107 vs 507 ± 429) were also not different between the NVP and control group ($p>0.05$). DHA $_{t½}$ was shorter with NVP (1.6 ± 0.8 vs 3.2 ± 1.4h; $P=0.004$). AQ data are being analyzed and will be presented. NVP-containing ART impacted important PK parameters of AS and DHA. These findings will inform future pharmacodynamic and clinical outcome studies of AS-AQ in HIV-infected patients receiving NVP-based ART.

1500

EVALUATION OF A NOVEL MOLECULAR MARKER FOR MONITORING ARTEMISININ RESISTANCE IN *PLASMODIUM FALCIPARUM* MALARIA

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The human malaria parasite *Plasmodium falciparum* has evolved resistance to most drugs. There is now evidence of reduced susceptibility to artemisinin derivatives and to Artemisinin Combination Therapy with delayed parasite clearance times. If artemisinin resistance spreads widely, it would threaten global malaria control. We still lack validated molecular markers for monitoring the resistance phenotypes. Using genome-wide strategies in the rodent malaria parasite *P. chabaudi* our group identified a mutation in a clathrin mu adaptor gene (pccmu) that arose along with

artemisinin resistance. In order to investigate the possible contribution of this candidate marker to artemisinin resistance in the human malaria parasite *P. falciparum* we screened the DNA sequence of the equivalent orthologue, pfcmu, for genetic polymorphisms. We have studied field isolates from an ACT clinical trial in Burkina-Faso that were tested *in vitro* for their response to artemisinin derivatives and other antimalarial drugs. We have also evaluated pre- and post- treatment samples from an *in vivo* ACT clinical trial carried out in Kenya. Genetic polymorphisms in pfcmu were analysed for association with various measured endpoints in the two trials that might indicate a drug resistant parasite phenotype. Our preliminary results indicate that mutations in this adaptor protein subunit may be associated with varying degrees of *in vitro* and *in vivo* responses to artemisinin derivatives, quinine and lumefantrine. We propose this gene should be evaluated further as a potential molecular marker of artemisinin resistance.

1501

PHARMACODYNAMICS OF CURRENT AND NOVEL ARTEMISININ COMBINATION THERAPIES AGAINST ARTESUNATE RESISTANT OR SENSITIVE *PLASMODIUM BERGHEI*

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Effective treatment of *Plasmodium falciparum* is threatened by signs of emerging resistance to artemisinins, the linchpin of Artemisinin-based Combination Therapies (ACTs) that have been widely adopted throughout malaria-endemic regions. Attempts to define resistance, in isolates from patients that showed delayed parasite clearance times following ACT therapy, have yet to replicate an artemisinin-resistant phenotype and no definitive genetic target has been identified. Using a *P. berghei* model, we have developed pharmacodynamic profiles of four existing ACTs (dihydroartemisinin-piperaquine, artesunate-amodiaquine, artesunate-mefloquine and artemether-lumefantrine), as well as the artesunate-pyronaridine ACT under clinical evaluation, generated against drug-sensitive and artesunate-resistant parasites. These profiles are developed from 30-day studies and assess the efficacy of a 3-day dosing regimen for each combination as well as efficacy of modifications to treatment frequency and concentration. All regimens are 3-days in duration and include each ACT component as monotherapy given once daily, ACT [1x] once daily, ACT [1x] split into two daily doses, ACT [1.5x] once daily and ACT [1.5x] split into two daily doses. Results to date elucidate differences in contribution to treatment between components of ACTs and suggest pairings and treatment schedules that are maximally effective against artesunate-resistant *P. berghei*. Furthermore, our data suggest cross-resistance between artesunate and the partner drugs amodiaquine, mefloquine and piperaquine, as well as possible antagonism in the artesunate-amodiaquine and artesunate-mefloquine combinations. These findings imply that some current pairings are at higher risk of treatment failures than others. Integration of these data with ongoing pharmacokinetic and genetic studies should shed light on drug mode of action and mechanisms of resistance in *P. berghei*, and help guide selection of suitable ACTs in areas where artemisinin resistance is a concern.

1502

EFFICACY OF ARTEQUICK (ARTEMISININ-PIPERAQUINE) AND COARSUCAM (ARTESUNATE-AMODIAQUINE) IN THE TREATMENT OF *PLASMODIUM FALCIPARUM* MALARIA IN VIETNAM

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Recent reports of artesunate resistant or tolerant *Plasmodium falciparum* malaria in western Cambodia is of immense concern as the fast acting artemisinins combined with the longer elimination half-life drugs such as mefloquine are now recommended worldwide for first-line treatment of uncomplicated *P. falciparum* malaria. In light of this information there is an urgent need to evaluate artemisinin combination therapies (ACTs) in neighbouring countries to Cambodia to determine whether reduced ACT susceptibility has developed with increase failure rates and prolonged parasite clearance times. The objective of the present study was to compare the efficacy of fixed-dosed combinations of artemisinin-piperaquine (Artequick®) and artesunate-amodiaquine (Coarsucam®) for the treatment of *P. falciparum* malaria in south central Vietnam. In an open-labelled, randomized clinical pilot study 128 patients (children aged 6-14 years, n=68, adults aged 15-60 years, n=60) were allocated either a 2-day course of either Artequick® (~2.8 mg/kg artemisinin plus ~16.9 mg/kg of piperaquine per day) or a 3-day course of Coarsucam® (~4.5 mg/kg of artesunate plus ~12.3 mg/kg of amodiaquine per day), with a follow-up period of 42 days. Both ACTs were well tolerated, with no obvious drug associated adverse events. Parasite clearance times ranged between 12 and 60 h for both treatment groups. Of the per-protocol population, 50 patients were on Artequick® (24 children, 26 adults) and 51 were on Coarsucam® (25 children, 26 adults). The PCR genotype corrected cure rate at day 42 was 98% for both ACTs. This study showed that the two ACTs were highly efficacious in the treatment of *P. falciparum* malaria in adults and children. Further studies of the ACTs are warranted in different regions of Vietnam to determine the nationwide effectiveness of the two ACTs.

1503

MITIGATION OF ARTEMISININ RESISTANCE IN AFRICA: WHAT SHOULD BE DONE NOW?

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Artemisinin resistance in *falciparum* malaria has emerged in Western Cambodia and may have spread westward, an ominous repetition of the spread of resistance to chloroquine over fifty years ago, and later to sulfadoxine-pyrimethamine. This poses a major global public health threat, with the greatest potential effects in sub-Saharan Africa where disease burdens are greatest and systems for drug resistance monitoring and containment are weakest. We do not fully understand the molecular mechanisms underlying artemisinin resistance. The only associated phenotype observed in South East Asia is a decreased parasite clearance rate, not yet seen in Africa. Artemisinin resistance fits the International Health Regulations (2005) definition of a public health emergency of international concern - it is serious, unusual, and has the potential to spread. Most African countries now recommend artemisinin

combination therapy (ACT) as first line regimen for uncomplicated malaria. Unfortunately, this policy shift has resulted in the demise of systematic drug resistance surveillance; sub-regional networks have very limited activities due to lack of funding and changing technical requirements for monitoring ACT efficacy. Well-defined early warning and detection systems need to be established. We will present strategies that should be urgently adopted by Africa, including: reactivation of regional surveillance networks with clear roles; data sharing to facilitate pooled analyses to identify rare observations; drug resistance risk factor modeling to bench mark baselines; and the development and validation of new tools for monitoring resistance, antimalarial drug pressure, drug quality and inappropriate drug usage.

1504

PILOT OF HOME-BASED MANAGEMENT OF MALARIA WITH RAPID DIAGNOSTIC TESTS AND ARTEMISININ-BASED COMBINATION THERAPY IN RURAL SENEGAL

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Malaria remains a major cause of morbidity and mortality in Senegal, and access to care is challenging in part due to the remoteness of many communities. Senegal introduced artemisinin-based combination therapy (ACT) in 2006, and diagnosis based on rapid diagnostic tests (RDTs) in 2007, resulting in 85% of suspected cases in the public health sector being tested in 2009. To expand access to case management based on parasitologic diagnosis, in 2008 Senegal introduced a pilot for home-based management with RDTs and ACTs in 20 remote villages >5 km from the nearest health post. Village members chose a volunteer home-based care provider (HCP) from the community, who received three days of classroom training on malaria case management, followed by a 15 day practical in the nearest health post. The HCPs were then officially installed and given a kit including RDTs, ACTs, a case record book, and stock management tools. The chief nurse at the nearest health post was responsible for the practical training and monthly supervision. Health officials at district, regional, and national levels also performed supervision visits. During the pilot, 869 (93%) of 939 patients were tested by RDT, of whom 290 (33%) were positive and all reported successfully treated. Of 525 cases referred, there were 521 negative tests, two pregnant women, one infant <2 months, and one severe case. There were no deaths among these patients. A multi-dimensional evaluation was conducted, including a household survey and interviews with health officials and community members. Perceptions were overwhelmingly positive among community members, community and traditional leaders, health post personnel, and district and regional health officials. They recommended greater involvement of health post chief nurses in the training, broadening HCP roles to include illnesses such as diarrhea, pneumonia, TB and malnutrition, reinforcing supervision and support to the HCP, and extension of this strategy to all unserved, remote villages in Senegal. Based on these recommendations, the pilot was expanded to 408 villages by the end of 2009 and 861 villages by the end of 2010.

1505

ARTEMISININ RESISTANCE ASSOCIATED WITH PFMDR1 COPY NUMBERS AND ANTI-OXIDANT ACTIVITY IN THE *IN VITRO* SELECTED PARASITES LINES

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To select an artemisinin resistant line in the laboratory, we have subjected *Plasmodium falciparum* Dd2 strain to dihydroartemisinin (DHA) selection with step-wise increments of drug concentrations over 13 months. Two lines (Art1 and Art2) were obtained more than 20-fold increase in IC_{50} to DHA. However, the resistance phenotype was unstable and the parasites could regain susceptibility to DHA after three months of culture in the absence of the drug selection pressure. Phenotype analysis showed that the resistant parasite displayed cross-resistance to a number of the commonly used antimalarial drugs. No mutations were detected on the reported drug resistant markers, but we identified increased copy number of *P. falciparum* multidrug resistance 1 (pfmdr1) gene associated with artemisinin resistance. To detect other potential changes in the selected parasites, we performed microarray analysis and detected increased expression of genes involved in redox metabolism. Collectively, this study suggests that selection of artemisinin resistance under the laboratory conditions may be associated with multiple mechanisms.

1506

ARC3: DETECTING RECENT POSITIVE SELECTION IN ARTEMISININ RESISTANT MALARIA PARASITES

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Following reports of poor clinical responses to artemisinin combination therapy (ACT) on the Thailand-Cambodia border, the Artemisinin Resistance Confirmation, Characterization, and Containment (ARC3) pilot

project was initiated to characterize the clinical, *in vitro* and molecular basis of artemisinin resistance in Southeast Asia. Four clinical trials of artesunate curative therapy were conducted at two sites in western Cambodia where emerging resistance was suspected; one site on the Thailand-Myanmar border, where prolonged parasite clearance times following artesunate-mefloquine treatment had also been reported; and in Bangladesh, where ACTs have not been used extensively and resistance was not suspected. Parasites collected during these trials were genotyped at approximately 8,000 single nucleotide polymorphisms (SNPs) using a molecular inversion probe SNP chip specific to *Plasmodium falciparum*. Genotyping data from 198 samples were used to detect genomic regions under recent positive selection. Each SNP was scored using three Extended Haplotype Homozygosity measures including the Long-Range Haplotype test, Integrated Haplotype Score, and Cross-Population Extended Haplotype Homozygosity. SNPs were also evaluated on their inter-population Fst values using a sliding window approach. The top 5% of loci from each statistic were ranked and re-scored. A comprehensive list based on the combined rank score revealed a number of novel regions possibly under recent positive selection, as well as previously described regions containing resistance genes such as *pfcrt* on chromosome 7 and *dhfr* and *dhps* on chromosomes 4 and 8, respectively. Regions identified as being under recent positive selection will be compared to regions identified in a genome-wide association study performed using the same sample set, and potential candidate genes within those regions will be discussed.

1507

COMPARATIVE EFFICACY AND ACCEPTABILITY OF ARTEMETHER-LUMEFANTRINE *VERSUS* DIHYDROARTEMISININ-PIPERAQUINE IN KENYAN CHILDREN WITH UNCOMPLICATED *FALCIPARUM* MALARIA

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Artemisinin-based combination therapies (ACTs) have become the cornerstone for the treatment of uncomplicated *falciparum* malaria worldwide. In Africa, artemether-lumefantrine (AL) and artesunate-amodiaquine are the most widely used ACTs. AL has been the first-line treatment for uncomplicated malaria in Kenya since 2006. Despite not yet receiving World Health Organization (WHO) prequalification, dihydroartemisinin-piperaquine (DP) has recently been adopted as a second-line treatment in Kenya. This was an open-label, randomized, comparative trial in children aged 6-59 months with uncomplicated *falciparum* malaria conducted in Western Kenya. Parasite clearance rate, sensitivity to and acceptability of AL and DP were monitored. In total, 466 children were enrolled in the study; they were hospitalized for 3 days for observed treatment and 72-hour parasite kinetic monitoring, and actively followed up at scheduled visits after discharge from hospital on Days 7, 14, 28 and 42. Hemoglobin levels were assessed on Days 0, 14, 28 and 42. Genotyping for determining treatment outcome was performed on Day 0 and any other day the study participant had a recurrence of parasitemia. The study drugs were administered by the parent/guardian under the observation of a study team member. At discharge from hospital, a questionnaire on the acceptability of the study drug was administered to the parent/guardian. The findings of this study on the parasite clearance rate, sensitivity to and acceptability of AL and DP in the treatment of uncomplicated malaria in Western Kenya will be discussed following completion of data analysis.

1508

SAFE AND EFFICACIOUS ARTEMISININ-BASED COMBINATION TREATMENTS FOR AFRICAN PREGNANT WOMEN WITH MALARIA

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Although malaria is the most important human parasitic disease, few studies with antimalarial drugs have been carried out in pregnant women. Pregnant women are a high-risk group requiring effective antimalarials but they are systematically excluded from clinical trials for fear of teratogenicity and embryotoxicity. This has complicated evidence-based recommendations for the prevention and treatment of malaria during pregnancy. A multicentre (Burkina Faso, Ghana, Malawi and Zambia), non-inferiority trial on the safety and efficacy of four artemisinin-based combinations for the treatment of *P. falciparum* malaria in pregnant women has recently started within the framework of the Malaria in Pregnancy Consortium and the financial support of both the European and Developing Countries Clinical Trials Partnership (EDCTP) and the Gates Foundation. Pregnant women in the second or third trimester of gestation and with a confirmed malaria infection are randomised to amodiaquine-artesunate, artemether-lumefantrine, dihydroartemisinin-piperaquine or mefloquine-artesunate. They will be followed up weekly until day 63 post-treatment and then monthly until 4-6 weeks and one year post-delivery. Explanatory variables for failure, i.e. drug levels and *in vitro* resistance of local malaria parasites are also collected. A total of 870 patients will be recruited to each treatment based on 290 patients in each treatment group in each country (i.e. a total centre sample size of 870 patients), adding up to a total study sample size of 3480 patients. A 3-arm trial using a "balanced incomplete block design" allow the treatments to be distributed in a way to allow a head-to-head comparison and the establishment of relative value of the treatment according to a series of outcomes. The primary end points are treatment failure (PCR adjusted) at day 28 and the safety profiles including significant changes in relevant laboratory values. More than 800 patients have been recruited so far. Results are expected by 2013.

1509

PARASITE CLEARANCE OF ARTEMISININ-CONTAINING REGIMENS IN THE TREATMENT OF UNCOMPLICATED MALARIA: A SYSTEMATIC REVIEW OF PUBLISHED TRIALS FROM 2000 TO 2010

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The deployment of artemisinin-based combination therapies (ACTs) for the treatment of acute uncomplicated *falciparum* malaria has become a key strategy for malaria control. The objective of this study is to review the recent trends in clinical trials of artemisinins and document parasite clearance times. Publications reported in PubMed, EMBASE, GH and the Cochrane Libraries between January 2000 and December 2010 were searched for the following key words: malaria, plasmodium, *falciparum*, *vivax*, ovale, malariae, knowlesi and anti-malarials. 1520 abstracts were indentified and 223 full text articles retrieved. In total 60% (n=96) of 160 clinical trials identified were conducted in Africa and 34% (n=54) in Asia.

Of the African countries, Nigeria hosted the maximum number of trials (n=14), whereas in Asia Thailand was the leading country (n=22). In total 138 (86%) studies were randomized and they included 309 treatment arms with an ACT given over 1-7 days. The duration of follow up was at least 42 days in 44 (28%) studies, 28 days in 107 (67%) studies and less than 28 days in 8 (5%) studies. A total of 47,855 patients were allocated an artemisinin-containing regimen in 160 trials with samples size ranging from 30 to 1553. The quality of clinical trials (as gauged by randomization, duration of follow-up, sample size) improved significantly in the second half of the decade compared to the first. The proportion of patients remaining parasitaemic at 48 and 72 hours could be determined in 125 and 114 treatment arms, respectively. Following initiation of therapy the median proportion of patients still parasitaemic at 48 and 72 hours was 6.4% (range 0-73) and 0.5% (range 0-78) respectively. There was no significant change in the proportion of patients remaining parasitaemic at 48 and 72 hours in studies conducted between 2000-2005 compared to 2006-2010 (p=0.093 and p=0.215 respectively). Standardised approaches are urgently needed to define with greater precision geographical and temporal trends in the parasite clearance following ACTs; this will require analysis of pooled data from individual records.

1510

IN VITRO CHARACTERIZATION OF THE METABOLISM AND DISPOSITION OF ARTESUNATE AND DIHYDROARTEMISININ

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IV Artesunate is currently approved for compassionate use in the US through the CDC, and is seen as a promising alternative to the only FDA-approved parenteral treatment of severe malaria in the United States, IV quinidine gluconate. While an effective antimalarial treatment, quinidine has potential serious cardiotoxicity. In support of efforts to obtain FDA approval for IV artesunate, our lab has performed a battery of *in vitro* metabolism and disposition assays of artesunate and its major metabolite, dihydroartemisinin (DHA). Artesunate demonstrated a short (<10 min) microsomal half-life in both human and mouse liver microsomes, while DHA exceeded the assay limits of 60 min. In human hepatocytes, the predominant metabolite of artesunate, as expected, was DHA, along with its glucoronide. These data were confirmed *in vivo* using clinical samples previously analyzed in this lab. In enzyme inhibition studies, artesunate demonstrated low potential for drug-drug interactions due to enzyme inhibition with any of the major CYP450s (1A2, 2C9, 2C19, 2D6, and 3A4) while DHA showed only moderate potential for inhibition of 1A2 and 2C9. Permeability was tested using MDR1-MDCK to assess cell permeation and potential as a Pgp substrate. These experiments showed medium/moderate permeability as compared to standards of known penetrability, and indicate a high potential as a Pgp substrate. The data support observations that AS and DHA are rapidly biotransformed and eliminated as the glucoronide of DHA. Evaluation of other potential metabolites is in progress.

1511

THE PHARMACOKINETICS AND PHARMACODYNAMICS OF A TWO- VERSUS THREE-DAY DOSE REGIMEN OF DIHYDROARTEMISININ-PIPERAQUINE IN PATIENTS WITH UNCOMPLICATED MALARIA IN NORTHERN CAMBODIA

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The combination of dihydroartemisinin (DHA) combined with long acting piperaquine (PIP) was recently adopted in Cambodia as the first line agent against both multi-drug resistant *Plasmodium falciparum* and *P. vivax*. In addition to well-documented safety and efficacy, a post-treatment prophylactic effect of DHA-PIP of up to 63 days has been reported, making it potentially valuable in malaria eradication efforts. While a 3-day course is widely recommended, the Cambodian military currently employs a 2-day regimen in order to improve compliance. The aim of this study was to compare the safety, tolerability and pharmacokinetic-pharmacodynamic relationships of a 2 versus 3 day dosing regimen of DHA-PIP using the same cumulative dose to establish the optimal regimen. From September 2010 to February 2011, in an open-label clinical trial, 80 patients with uncomplicated malaria were randomized 1:1 to receive 9 fixed-dose combination tablets (total dose 320mg of DHA and 2880 mg PIP), divided into either a 2 or 3 day course. Plasma piperaquine levels from all volunteers receiving DP were collected at pre-dose (time 0), 4, 24, 48, 72 hr, 7, 14, 21, 28, 35 and 42 days after the first dose, and on day of recurrence. Sixteen patients were diagnosed with P.f. (20%), 61 with P.v. (76%) and 3 with mixed species infection (4%). Uncorrected 42-day efficacy rates were not statistically significantly different between treatment groups by per protocol analysis - 89% for 2 days (95% CI = 76-96%) and 92% for 3 days (95% CI = 80-97%) of DP. Mean parasite clearance times were 11.1 hours for *P. vivax*, but 72.5 hours for *P. falciparum*. Piperaquine levels are currently being measured by liquid chromatography-mass spectrometry. Pharmacokinetic data will be presented, along with pharmacodynamic analysis of parasite clearance and PCR-corrected treatment efficacy, with particular attention to drug levels at the time of failure and post-treatment prophylactic effect.

1512

ARC3: ASSOCIATIONS BETWEEN CANDIDATE GENE POLYMORPHISMS AND PARASITE CLEARANCE RATE FOLLOWING TREATMENT WITH ARTEMISININS

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Nearly all malaria-endemic countries have replaced resistance-compromised antimalarial drugs with artemisinin-based combination therapy, the efficacy of which is also at risk due to the recent emergence of artemisinin-resistant *falciparum* malaria on the Thailand-Cambodia border. Without knowledge of the mechanism of action of the artemisinins, genome-wide approaches are being taken to identify loci associated with resistance to this class of drugs. In addition, candidate genes that have been reported in the literature as possibly being associated with artemisinin resistance are being evaluated. Full-length sequences were determined for three candidate genes in *Plasmodium falciparum* infections in four clinical trials of 7-day curative artesunate therapy conducted as part of the Artemisinin Resistance Confirmation, Characterization, and Containment (ARC3) pilot project. The sequenced loci include: *pfmdr1*, a gene associated with resistance to other antimalarial drugs, *pfserca*, a gene encoding a sarco/endoplasmic reticulum calcium-dependent ATPase, and *pfubp1*, coding for an ortholog of a deubiquitinating enzyme associated with artemisinin resistance in rodent malaria. Polymorphisms in each of these genes, as well as variations in copy number estimates of *pfmdr1*, were evaluated for associations with parasite clearance rate and will be presented in the context of results from genome-wide analyses from the same studies. Strategies for prioritization of newly identified candidate genes will be discussed.

1513

EFFICACY OF DIHYDROARTEMISININ PLUS PIPERAQUIN COMPARED TO AMODIAQUIN PLUS SULFADOXIN/ PYRIMETHAMIN IN SEASONAL IPT OF MALARIA IN CHILDREN IN A RURAL AREA OF BOBO-DIOULASSO (BURKINA FASO)

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Intermittent preventive treatment (IPT) is a promising malaria control strategy. Optimal regimen and the best option for his delivery remain unclear. The long elimination half-life of Piperaquine (PQ) makes its coformulation with dihydroartemisinin (DHA), suitable for IPT. To assess the effectiveness of DHA + PQ compared to AQ + SP in seasonal IPTc, 1500 children aged 3-59 months were randomized to receive DHA+PQ or SP+AQ once a month from August to October 2009. A comparator arm with 250 children was also recruited and followed up in parallel. A cross sectional survey was led at the end of transmission season. Primary endpoints were the incidence of clinical malaria attacks. Coverage of all three courses of IPT and compliance of children to daily doses of IPT was similar in the two arms. 83% of children in each arm received at least the first dose of the 3 courses of IPT and 99% to 100% of them completed the entire three doses regimen. A total of 328 episodes of clinical malaria with any parasitemia (incidence rate (IR) of 0,047(95%IC=[0,042 to 0,052] episodes per child day at risk (CD)) was observed in the control arm compared to 205 (IR of 0,003(95% IC= [0,002 to 0,003] episodes per CD) in DHA+PQ arm and to 147 (IR of 0,002(95% IC= [0,001 to 0,002] episodes per CD) in SP+AQ arm, indicating a protective efficacy (Pe) of 93% (95% IC= [92 to 94] p<0.0001) for DHA+PQ versus 95% (95% IC = [94 to 96] p= 0.0001) for SP+AQ. Children in SP+AQ arm were better protected against malaria attacks than those in DHA+PQ arm (Pe = 28% (95% IC=[10 to 42] p= 0.003). At the end of the transmission season, only SP + AQ retained a substantial efficacy (Pe = 62% (95% CI = [32-78] p= 0.001) against malaria attacks. The prevalence of parasitaemia was reduced by 67%, in the two treated arms. The prevalence of anemia (Hb< 10g/dl) was similar in DHA+PQ (40%) and SP+AQ (35.34%) arms with a respective Pe of 17% (95% CI = [3 to 29], p = 0.026), and 27% (95% CI = [14 to 27], p = 0.001). The mean Hb in SP + AQ arm was significantly higher than the DHA + PQ group and control group. In conclusion, SP+AQ was the most efficacious for IPTc in our study but its use must be reserved to area with low-level SP resistance otherwise DHA+PQ would be a suitable alternative.

1514

A COMPARISON BETWEEN *EX VIVO* AND *IN VITRO* (CRYOPRESERVED + CULTURE ADAPTED) ARTEMISININ SUSCEPTIBILITIES FOR *PLASMODIUM FALCIPARUM* ISOLATES FROM WESTERN CAMBODIA

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Artemisinin derivatives are the most potent first line antimalarial drug in use today. However, recent studies along the Thai-Cambodian border conducted during the past 5 years demonstrate that *Plasmodium falciparum* may be becoming resistant to the artemisinin class. Since clear genetic markers of artemisinin resistance have not been discovered, *in vitro* parasite drug susceptibility testing may be helpful to identify resistance trends. In many *in vitro* drug sensivity studies, samples are collected from untreated subjects in the field and cryopreserved to facilitate sample transport. Because such samples must be thawed and recultured, the IC50 measured after recovery may diverge from the *ex vivo* IC50. During a dose ranging randomized open label clinical trial of a 7 day course of mono-therapy artesunate (Artemisinin Resistance in Cambodia - 2; 2008-2009) parasite samples were analyzed *ex vivo*, without cryopreservation or culture adaptation, for susceptibility to dihydroartemisinin, artesunate, and other anti-malarial drugs using a histidine-rich protein-2 (HRP2) ELISA method. IC_{50} values obtained for the artemisinins (DHA and AS) did not correlate with treatment outcome. However, the values were two-fold higher than those obtained in an earlier study (Artemisinin Resistance in Cambodia - 1, 2006-2007) conducted at the same location. This increase suggests that *falciparum* parasites in this location are progressively more tolerant to the artemisinin class of antimalarials. However, we and others have not determined if this tolerance is maintained in culture in the absence of drug pressure. We will report the *in vitro* analysis of cryopreserved parasites and compare them to matching uncryopreserved *ex vivo* results. Parasite susceptibility to other anti-malarial drugs and change in parasite genetic constituent will also be described. These results will be helpful to inform future surveillance

investigating the problem of artemisinin resistance, particularly whether resistance IC50 phenotypes can be preserved through cryopreservation and recovery.

1515

INCREASE IN EX VIVO IC_{50} VALUES TO ARTEMISININS FROM A SEVEN-DAY ARTEMISININ MONO-THERAPY TRIAL CONDUCTED IN WESTERN CAMBODIA IS NOT DUE TO INOCULUM EFFECT

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Studies along the Thai-Cambodian border conducted during the past 5 years demonstrate that *P. falciparum* may be developing resistance to the artemisinin class of antimalarial drugs, the last first-line antimalarial compounds in use today. Persistence of parasitemia beyond 72 hours has been proposed as a parasitological marker of diminished artemisinin susceptibility. During a dose ranging randomized open label clinical trial of a 7 day course of mono-therapy artesunate (Artemisinin Resistance in Cambodia - 2; 2008-2009) parasite samples obtained pre-treatment (on Day 0) were analyzed ex vivo, without cryopreservation or culture adaptation, for susceptibility to dihydroartemisinin, artesunate, and other anti-malarial drugs using a histidine-rich protein-2 (HRP2) ELISA method. IC50 values obtained for the artemisinins (DHA and AS) did not correlate with treatment outcome. However, there was a statistically significant relationship between parasite clearance time greater than 72 hours (PCT > 72hrs) and elevated IC50 values for DHA ($p<0.003$). Previous reports have implicated an inoculum effect, or the parasitemia of the isolate, as a possible reason for elevated IC50 values against the artemisinins. Here we argue that the association between DHA IC50 value and PCT>72hrs may not be solely due to inoculum effect, and may represent the early stages of *in vitro* resistance to the artemisinins.

1516

A RANDOMIZED CLINICAL TRIAL OF ARTEMISININ VERSUS NON-ARTEMISININ-BASED COMBINATION THERAPY OF UNCOMPLICATED MALARIA IN MALI

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Plasmodium falciparum resistance to artemisinin has been reported in South-East Asia. The potential spread of this resistance is real and makes a search for alternative non-artemisinin-based malaria therapy urgent. We tested the hypothesis that sulphadoxine-pyrimethamine plus artesunate (SP+AS) is as efficacious as sulphadoxine-pyrimethamine plus amodiaquine (SP+AQ) in the treatment of uncomplicated *Plasmodium falciparum* malaria. From August to December 2004 and July to December 2005, we conducted a randomized single-blind trial of SP+AS and SP+AQ in two localities in Mali. Parasite genotyping by polymerase chain reaction (PCR) was used to distinguish new from recrudescent *P. falciparum* infections. We recruited a total of 610 children aged 6 to 59 months, with uncomplicated *P. falciparum* malaria and followed them for 28 days to assess treatment efficacy. Baseline characteristics were similar in both treatment groups. The analysis revealed no early therapeutic failures (ETF) in both arms; late clinical failures (LCF) were 1.7% for SP+AS (n=5) vs. 0% SP+AQ (n=0) and late parasitological failures (LPF) were 3.4% SP+AS (n=10) vs. 1.4% SP+AQ (n=4) ($p>0.05$). We observed a rate adequate clinical and parasitological response (ACPR) of 94.9% and 98.6% for SP+AS and SP+AQ respectively ($p=0.98$). Based on *msp2* analysis, the rate of re-infection was respectively 4.1% and 1.4% for SP+AS and SP+AQ. After molecular correction, we obtained an ACPR of 99% for SP+AS, and 100% for SP+AQ ($p=0.98$). Sulphadoxine-pyrimethamine plus amodiaquine therapy is as efficacious as sulphadoxine-pyrimethamine plus artesunate in the treatment of uncomplicated *P. falciparum* malaria in Mali.

1517

STUDY OF *PLASMODIUM VIVAX* DUFFY BINDING PROTEIN (DBP) OF EASTERN INDONESIAN ISOLATES

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Erythrocyte invasion by malaria parasites is a series of complex processes and specific interaction between parasite ligands and host receptors play a key role. *Plasmodium vivax* uses the critical ligand Duffy Binding Protein (DBP), to bind its host receptor, Duffy Antigen Receptor for Chemokines (DARC), and initiate the formation of tight junction between merozoite and erythrocyte essential for invasion. The central domain of Domain II of DBP serves as the critical binding motif to the DARC. However, the genetic diversity of PvDBPII is a major obstacle to vaccine development. In order to develop a rational vaccine, more information about the sequence polymorphisms of PvDBPII is required, from diverse geographical regions. The current study aims to determine the extent of polymorphisms of PvDBPII from Timika, West Papua, Indonesia and to examine the relationships of different dbp alleles with published sequences through phylogenetic analysis. 95 *P. vivax* isolates were collected from the hospital-based study in Timika from 2006-2008. These DNA samples were subjected to PCR-RFLP using Pvmsp-3α to assess its genetic polymorphisms. Some samples were selected and further subjected to cloning and sequencing to examine the PvDBPII sequence polymorphisms. A phylogenetic tree was constructed based on the PvDBPII sequences obtained in this study and combined with the published sequences using Bayesian inference. A total of 101 DBP point-mutations were found from the Timika isolates. Mutations mainly occurred in the critical binding motif of PvDBPII. Most mutations (81%) were non-synonymous; altering amino acids sequence and only about a fifth (23%) were synonymous. R308S, K371E, D384G, R390H, N417K, L424I, W437R, I503K were the highest frequency polymorphisms seen. In total, about 51 different haplotypes of PvDBPII were observed. More haplotypes might be found if more clones are sequenced. The phylogenetic tree showed that Timika PvDBPII isolates clustered together with isolates from other geographic regions, including Brazil, Sri Lanka, and Papua New Guinea. Although the PvDBPII sequences of Timika isolates are quite diverse as shown by the number of haplotypes detected and the topology of the phylogenetic tree, the similarities between those sequences are still high. These findings certainly contribute to the development of PvDBPII vaccines against *P. vivax* infections, and thus demonstrate its public health significance.

1518

FREQUENCIES OF SOME HUMAN GENETIC MARKERS AND RELATIONSHIP WITH *PLASMODIUM FALCIPARUM* AND *P. VIVAX* MALARIA IN COLOMBIA

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Malaria in Colombia is highly endemic in the Northwest, Pacific Coast and Amazon regions. Despite the high frequencies of *Plasmodium falciparum* infection, severe or fatal malaria cases are rare. Out of 79.909 malaria cases (72% *P. vivax* - 27% *P. falciparum*) reported in 2009, only 307 were severe (1,4% of *falciparum* cases) with 0,04% fatality rate. Some proposed that *P. falciparum* shaped the distribution of ABO blood groups in humans with some groups being protective against severe disease. The Duffy blood group A has marked selective and sickle-cell disease, G-6-PD deficiency a more discrete one. Aimed at understanding the blood genetic factors underlying the apparent protection to development of severe disease within a *falciparum* malaria infected community, we explored the ethnic background, blood types and *Plasmodium* infection in La Italia on the Pacific Coast. A descriptive, cross-sectional study in a deprived and mainly rural region was conducted. Sample sizes were Afro-American, 73; Amerindian (Emberá), 74 and Mestizo, 171. Presence of *Plasmodium* infection was assessed by thick smear, ABO and Rh were determined by agglutination and Duffy status by PCR and RFLP. For ABO, 69% were O, 21% A, 8% B and 2% AB. A significant association was observed for ABO status and ethnicity: 100% of Amerindias were group O. Similarly, Duffy genotypes were significantly associated to ethnicity (p=0,003). Expression of Rh was confirmed in 97-99% of all subjects, regardless of the ethnic background. For Duffy, the C/C,A/A diplotype carriers were exclusively infected by *P. falciparum*. At locus 131, the frequency of the G allele was 0,30 in Amerindians and the A allele was 0,69 in Afrocolombians. *Plasmodium* infection was confirmed in 17% (*falciparum* 2,2: 1 *vivax*), 33% were asymptomatic and 3,1% of *falciparum* cases developed severe infection. In conclusion, a very high frequency of group O was confirmed in a locality where *falciparum* infection is frequent. High endogamy and population differentiation was confirmed after Duffy genotyping. Infection by *P. vivax* was not detected in CC(FY-/FY-) individuals.

1519

CRYOPRESERVED *PLASMODIUM VIVAX* AND RETICULOCYTES CAN BE USED FOR INVASION AND SHORT TERM CULTURE

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The development of a *Plasmodium vivax in vitro* culture system is critical for the development of new vaccine, drugs and diagnostic tests. Though short term cultures have been successfully set up, their reproducibility in laboratories without direct access to *P. vivax* patients has been limited by the need of fresh parasites isolates. We have explored the possibility of using both frozen parasite isolates and frozen reticulocytes to perform invasions and start a short term culture. More than 50 invasion tests were performed. Invasion could be performed with similar efficiency for any of the combination (fresh/frozen reticulocytes and *P. vivax* isolates) used. This method should be easily replicated in laboratories outside endemic areas and can substantially contribute to the development of a continuous *P. vivax* culture.

1520

RETICULOCYTES DERIVED FROM HEMATOPOIETIC STEM CELLS CAN BE SUCCESSFULLY CRYOPRESERVED TO BE USED FOR *PLASMODIUM VIVAX* INVASION TESTS

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The differentiation of hematopoietic stem cells (HCS) into reticulocytes has been previously described as potentially interesting for the development of the *Plasmodium vivax in vitro* culture. Nevertheless, the need of using both freshly derived reticulocytes and fresh *P. vivax* isolates remained an obstacle, particularly for laboratories located in non-endemic countries. We describe a new method for the cryopreservation of reticulocytes produced after 14 days of culture from HSCs. Invasions assays have been carried out with both frozen isolates of *P. vivax* as well as laboratory strains of *P. falciparum*. Cryopreserved *P. falciparum* and *P. vivax* isolates could equally invade fresh and cryopreserved reticulocytes. This new technique represents an important advance towards the establishment of a continuous *P. vivax* culture as it allows the storage of large quantities of reticulocytes to be later used for the invasion.

1521

BIOMECHANICAL AND NANOSTRUCTUAL CHANGES TO THE *PLASMODIUM VIVAX* INFECTED RED BLOOD CELL MEMBRANE

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The infection of reticulocytes by *Plasmodium vivax* results in significant changes to the infected red cell membrane (IRBCM). Most importantly, the *P. vivax* IRBCM becomes highly deformable relative to membranes of the reticulocyte and mature normocyte. We present a range of data clearly demonstrating these biomechanical changes to the IRBCM using single cell and (micopipette, microfuidics) and cell population measurements (LORCA). Although the mechanism behind these changes is not fully understood, increased deformability would aid *P. vivax* avoid splenic clearance. In addition to these biomechanical changes we examined the development of 'caveolae' on the surface of the IRBCM in staged *ex vivo* cultures of *P. vivax* IRBCs, using TEM, SEM and Atomic Force Microscopy. We show that caveolae-like depressions found in the host cells (reticulocytes) and *P. vivax* IRBCs are morphologically and biologically distinct, thus unrelated. We also show that 'Schüffner's dots', a classical diagnostic feature of *P. vivax*, is associated with parasite derived vesicle complexes and not caveolae.

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DETECTION OF *PLASMODIUM VIVAX* PRE-ERYTHROCYTIC STAGE USING A ROLLING CIRCLE AMPLIFICATION (RCA) ASSAY

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A rolling circle amplification (RCA) assay was developed to detect the pre-erythrocytic stage in liver cells infected with *Plasmodium vivax*. The circular probe (98bp) in combination with a fluorescence DNA detector probe was designed to have a complementary sequence (20bp in length) to malaria 18S rDNA at the 5' and 3'ends of the probe. The RCA technique was previously developed and applied to detect several pathogens in order to increase detection sensitivity in clinical samples. The target sequence amplification step uses phi 29 or Bst enzymes to enhance the intensity of the detection signal in the RCA assay. Our results showed that the *in vitro* RCA assay for the detection of *P. vivax* resulted in a fluorescent signal significantly higher relative to the signal resulting from the real-time PCR method (8.0 versus 0.5 fluorescent intensity). However, the RCA assay required more time and additional steps when compared to the real-time PCR assay. *In situ* RCA was established using an infected blood smear (thick film) collected from a *P. vivax*-infected patient and with *P. berghei*-infected HepG2 cells collected at 48h post-infection. An infected cell in a blood smear generated a strong RCA signal although the number of positive cells was lower than that observed with immunofluorescence staining using a heat shock protein 70 (HSP70) monoclonal antibody. In a liver cell culture infected with *P. berghei*, the infected cells generated a strong RCA signal and all infected cells were confirmed by an immunofluorescence signal. In conclusion, the RCA technique can detect *P. vivax* in infected liver cells even where there are low infection rates and therefore such a technique will allow researchers to better understand the biology of *P. vivax* in liver cells.

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ASYMPTOMATIC *PLASMODIUM VIVAX* INFECTIONS IN SOUTHERN MINDANAO, PHILIPPINES: A CHALLENGE TO MALARIA ELIMINATION

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The Philippines is among the 39 countries progressing towards malaria elimination. The country shifted to artemether-lumefantrine in 2009, dispensed insecticide-treated nets, and conducted indoor residual spraying to address malaria in 58 out of 80 endemic provinces. However, in low transmission settings asymptomatic infections especially with *Plasmodium vivax* can hamper elimination efforts when parasitemia is too low for detection by microscopy in health clinics. In this study, we compared the performance of pLDH/HRP2-based rapid diagnostic test (RDT) for *P. falciparum* and *P. vivax* with PCR in detecting asymptomatic infections. Using the RDT in a cross-sectional survey in Sarangani Province to screen 930 participants (aged 12 mos. and above), we detected one *P. vivax* infection (a prevalence of 0.10%) and 10 *P. falciparum* infections (1.08%) all of whom were asymptomatic upon presentation. We also collected blood spots on filter paper for molecular and serological assays. To date, we have screened 700 out of 930 blood spots by PCR using species-specific primers for the 18SrRNA gene of *Plasmodium*. PCR results showed 7 participants (1%) were infected with *P. falciparum* (4 of whom were RDT negative) while 10 participants (1.4%) were infected with *P. vivax*, 9 of whom were RDT negative. Four of the 7 *P. falciparum*-infected individuals lived in a forested B'laan tribal community in Maasim, Sarangani which was 10-12 km from formal health systems, while 6 of the 10 *P. vivax*-infected individuals lived in a forest fringed T'boli tribe community in Kiamba, Sarangani. These infections would have been undetected and untreated as no clinical signs of malaria were evident. Our findings to date indicate pockets of ongoing malaria transmission despite current control measures, which hamper malaria elimination efforts; and the sensitivity of the Pf/Pv combination RDT is insufficient to detect asymptomatic *P. vivax* infections. On completion of PCR screening, we plan to calculate the comparative performance of the RDT versus PCR in diagnosing asymptomatic *P. vivax* infections in Southern Philippines. We will also complete serological assays for exposure to Pf/Pv infections on the same samples. Together with PCR and questionnaire data we aim to build a complete epidemiologic profile of malaria in Sarangani and guide intervention measures to halt transmission.

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A MALARIA DIAGNOSTICS TO BE USED IN THE FIELD: VISUALIZED LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) FOR DETECTION OF *PLASMODIUM VIVAX* INFECTION

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Loop-mediated isothermal amplification (LAMP) is a high performance method for detecting DNA and is of high potential usage in the molecular detection of infectious pathogens including *Plasmodium spp*. However, in most malaria endemic areas, which are often resource-limited, current LAMP methods are not feasible for diagnosis due to difficulties in accurately interpreting results with problems of sensitive visualization of amplified products, and the high risk of contamination resulting from the high numbers of amplified DNA sequences produced. In this study we establish a novel visualized LAMP method in a closed-tube system, and validate it for the diagnosis of malaria in a simulated field condition. A visualized LAMP method was established by the addition of a microcrystalline wax-dye capsule containing the highly sensitive DNA fluorescence dye SYBR Green I to a normal LAMP reaction prior to the initiation of the reaction. The wax remained intact during isothermal amplification, and released the DNA dye to the reaction mixture only when the temperature was raised to the melting point following amplification Soon after cooling down, the solidified wax sealed the reaction mix at the bottom of the tube, thus minimizing the risk of aerosol contamination. A total of 89 of field blood samples were collected on filter paper and processed using a simple boiling method for DNA extraction. This was then tested by the visualized LAMP method. Compared to microscopy, the sensitivity and specificity of LAMP were 98.3% (95% CI, 91.1% to 99.7%) and 100%, and were in close agreement with a nested PCR method. In conclusion, this novel, cheap and quick visualized LAMP method is feasible for malaria diagnosis in resource limited field settings.

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REAL TIME LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (REALAMP) FOR THE SPECIES-SPECIFIC IDENTIFICATION OF *PLASMODIUM VIVAX*

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Plasmodium vivax infections remain a major source of malaria-related morbidity and mortality. Early and accurate diagnosis is an integral component of effective malaria control programs. Conventional molecular diagnostic methods provide accurate results but are often resource-intensive, expensive, have a long turn around time and are beyond the capacity of most malaria-endemic countries. Our laboratory has recently developed a new platform called RealAmp, which combines loop-mediated isothermal amplification (LAMP) with a portable tube scanner real-time isothermal instrument for the rapid detection of malaria parasites. This method was initially tested using *Plasmodium* genus-specific primers, but species-specific diagnosis was not accomplished as there were no primer sets that gave consistent results. Here we describe a new primer set for the detection of *P. vivax*. The LAMP assay was designed using three pairs of amplification primers targeting a conserved DNA sequence unique to the *P. vivax* genome using an algorithm we have developed for genome mining. The amplification was carried out at 64ºC using SYBR Green intercalating dye for 90 minutes with the tube scanner set to collect fluorescence signals at 1-minute intervals. Clinical samples of *P. vivax* and other human-infecting malaria parasite species were used retrospectively to determine the sensitivity and specificity of the primers using the 18s ribosomal DNA based nested PCR as the gold standard. The new set of primers showed promising results in detecting laboratory-maintained isolates of *P. vivax* from different parts of the world. The time to amplification ranged from 18 to 49 minutes from the start of the reaction. The primers detected *P. vivax* in the clinical samples with 89.29% sensitivity and 100% specificity compared to the gold standard nested PCR method. The new primers also proved to be more sensitive than the published species-specific primers in detecting *P. vivax*. Further validation of this test using prospective testing in endemic countries will help deploy this tool for future field use.

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DETECTION OF ASYMPTOMATIC *PLASMODIUM FALCIPARUM, P. VIVAX* AND *P. MALARIAE* DURING LOW-TRANSMISSION SEASON IN THE HILL TRACTS OF BANGLADESH

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During the low transmission season many malaria infections are asymptomatic. To investigate how best to target asymptomatic individuals, an active randomized, population-based malaria surveillance was initiated by the JHMRI and ICDDR,B in two unions near hypoendemic Bandabarn in the Chittagong Hill Tracts of Bangladesh. The population of 20,000 with approximately 4,500 households was enumerated in a baseline census with GIS-mapping. Detection of *Plasmodium* species using microscopy, RDT and Real-time PCR was performed for the active and passive case detection. Microscopy, RDT, and real-time PCR in the active surveillance showed approximately 2% positive rates by microscopy or RDT in nearly 500 individuals. A real-time PCR assay detected the prevalence of 6%. The sensitivity of the RT-PCR in the 96-well format was increased to 10-100 parasites/µl with a glycogen/acetate DNA precipitation at low-speed tabletop centrifugation after column extraction. *P. vivax* and *P. malariae* were detected in less than 5% of the malaria-positive patient samples by RT-PCR. All the *P. falciparum* isolates were chloroquine-resistant PfCRT K76T genotype and atovaquone-sensitive PfCYTb 268Y by fluorescent TAQman probe analysis. A reverse transcriptase real-time PCR assay from dried blood on filter papers was able to detect gametocytes. HRP2 antigen detection by RDT persisted up to 28 days in more than 20% of malaria cases in a density-dependent fashion. The geometric mean parasitaemia was 227; 1,342, 5,412, and 10,716 for persistent RDT-positives through Day 0; Day 2; Day 7, and Day 28 respectively. Both microscopy and PCR detection diminished in a few days. Studies on malaria seropositivity rates are in progress. Most of the malaria-positive cases clustered in less than half of the population. Significant asymptomatic populations PCR-positive, RDT and microscopy negative malaria exist. The role of this sub-population in contributing to continuing transmission is being evaluated. Passive surveillance detected less than half of the malaria infections.

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EFFICACY OF CHLOROQUINE FOR TREATMENT OF *VIVAX* MALARIA IN CENTRAL CHINA

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Therapeutic efficacy studies allow measurement of the clinical and parasitological efficacy of medicines and the detection of subtle changes in treatment outcome when monitored consistently over time, which was considered the gold standard for determining antimalarial drug efficacy, and their results are the primary data used by national malaria control programmes to make treatment policy decisions. This study was to measure the efficacy of Chloroquine (CQ) for treatment of *vivax* malaria, and evaluate the incidence of adverse events.From June to October of 2008 and 2009, thirty-eight patients, who met the inclusion criteria, and infected with uncomplicated *vivax* malaria, confirmed by two qualified microscopists in Suining county of central China, were enrolled. CQ was administered at the dose of 25 mg base/kg body weight over 3 days(day 0,1,2), and after 28 days follow-up, primaquine was administered of 0.25mg/kg body weight, taken with food once daily for 14days.The follow-up consisted of a fixed schedule of check-up visits and corresponding clinical and laboratory examinations including thick and thin blood films for parasite count, auxiliary temperature and adverse events. Patients were classified as therapeutic failures or treatment success based on the assessments. It was observed that thirty-seven patients were treatment success over time, no fever and parasitemia were occurred on day 2 and only one patient with 200/ml parasitemia on day 3. Except the normal symptoms including headache, unwell, no severe adverse effect was reported. It was concluded that CQ is still effective and safe efficacy, there was no obvious data to support the CQ resistance to *Plasmodium vivax* at present. Nevertheless, according to the discovering in this study, the initiative diagnosis technique and method should be developed to detect the possible changing pattern and stage of CQ to *Plasmodium vivax*, to formulate recommendations and to enable the Ministry of Health and to make informed decisions about the possible need for updating of the current national antimalarial treatment guidelines. This study was approved by the national ethical committee, and all the patients signed the informed consent, all information regarding the patients was remaining confidential within the study team.

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STAGE SPECIFIC DRUG ACTIVITY OF CHLOROQUINE IN *PLASMODIUM VIVAX* MALARIA: IMPLICATIONS FOR *EX VIVO* DRUG RESISTANCE TESTING

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The emergence of chloroquine resistance in *Plasmodium vivax* has enhanced the need for greater understanding of the epidemiology of the disease, the mechanisms of drug resistance in these parasites, and effective case management. Historically the Schizont Maturation Test (SMT) has been used to monitor drug resistance in asexual stages of *P. falciparum*. Modifications are subsequently required when the test is applied to *P. vivax* due to the high diversity of life cycle stages present in blood samples taken from the peripheral circulation. In this study we analysed the results from 760 isolates assessed by the SMT to investigate how development time of the parasite is related to drug sensitivity. We confirm the previous hypothesis that chloroquine has a stage specific activity against *P. vivax*, and show that this stage specific activity may have profound consequences for the interpretation of the SMT. Using threshold models we show that increasing assay duration is associated with decreased effective concentration (EC50) values. EC50 values are also shown to be linked to the proportion of ring stages in the initial blood sample. We further demonstrate that assays with a duration of less than 34 hours (upper 95% CI 39 hours) should not be used to estimate EC50, nor an assay where the abundance of rings stage parasites in the initial sample collected at venipuncture does not exceed 66% (upper 95% CI 90%) of the total parasites. As late stage *P. vivax* parasites are insensitive to chloroquine, the starting composition of parasites in the SMT can dramatically affect the estimated EC50 values for chloroquine; the EC50 will be erroneously high if only resistant, late stage parasites are exposed. Application of this threshold modelling approach suggests that similar issues may occur for testing of resistance to amodiaquine and mefloquine. The statistical methodology that has been developed also provides a novel means of detecting stage-specific drug activity for new antimalarials.

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PVMDR1 MUTATION FOR GENETIC EPIDEMIOLOGY OF CHLOROQUINE RESISTANT *PLASMODIUM VIVAX*

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A mutation in *Plasmodium vivax* multidrug resistance 1 gene (*pvmdr1*) in codon 976 (Y976F) is reported to be associated with chloroquine (CQ) resistance and is recently used to monitor the distribution and frequency of the CQ resistant *vivax* malaria. In this study, we determined the mutation in codon 976 in the *pvmdr1*, using 28 *P. vivax* field isolates from the Philippines (6 isolates), South Korea (4 isolates), Papua New Guinea (PNG) (4 isolates), India (2 isolates), Indonesia (1 isolate), Thailand (1 isolate), Bangladesh (1 isolate), Nepal (1 isolates), China (1 isolate), Iran (1 isolate), Rwanda (1 isolate), Sudan (1 isolate), Comoros (1 isolate), Brazil (2 isolate) and Ecuador (1 isolate), collected in our National Center for Global Health and Medicine, Japan, from 1999 to 2011. The Y976F mutation was observed in the 13 isolates out of the 28 isolates (46%): 6 from the Philippines, 2 from PNG, 1 form India, 1 from Thailand, 1 form Rwanda, 1 form Sudan and 1 form Comoros. The other 15 isolates possessed the wild type (Y976). In the Philippines, CQ has been used for treatment of *vivax* malaria of which resistance against CQ has never been reported so far. However, all the Philippine isolates (collected in Palawan island in 2009) acquired the Y976F mutation. This finding suggests that CQ resistant *vivax* malaria will be emerging in the endemic area in the near future or that this mutation may not be critical to CQ resistance, at least in the Philippine population. On the other hand, in South Korea, all the 4 South Korean isolates of ours collected in 1999 showed the wild type (Y976) in the gene, but later in 2003 and 2007, 2 cases of CQ resistant *vivax* malaria were reported. The correlation of the Y976F genotype and the phenotype of *P. vivax* to CQ has to be further examined to evaluate the reliability of this molecular marker for the genetic epidemiology of *vivax* malaria.

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FIELD *EX VIVO* SENSITIVITY TESTING OF *PLASMODIUM VIVAX AND P. FALCIPARUM*: IMPORTANT CONSIDERATIONS AND A NEW FIELD BASED CYTOMETRIC METHOD

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The *ex vivo* sensitivity testing of antimalarials is an important adjunct to *in vivo* resistance testing. Except for the microscopic schizont maturation assay (MSMA), there are no standardised protocols for side-by-side comparison of the sensitivity profiles *of Plasmodium vivax* and *P. falciparum* clinical isolates. Here we present a practical cytometry based sensitivity assay that provides a viable field based alternative to the MSMA. We discuss a range of important confounders to any sensitivity assay utilising clinical isolates, including the presence of host leukocytes, which decreases antimalarial IC_{50}s, and the initial developmental stage of the parasite, which in the case of *P. falciparum* may result in the failure of H^3 Incorporation assays.

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FIRST REPORT FROM SOUTHERN PAKISTAN ON ALLELIC VARIANTS OF *PLASMODIUM VIVAX CIRCUMSPOROZOITE PROTEIN* (*PVCSP*) AND *MEROZOITE SURFACE PROTEIN1 (PVMSP1)*

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Plasmodium vivax is the prevalent malarial specie accounting for 70% of malaria cases in Pakistan.However,basic data on *P.vivax* genotypes is lacking from Pakistan.Studies have shown that for *P. vivax*, polymorphic genes coding for circumsporozoite protein *Pvcsp* and merozoite surface protein 1 *Pvmsp1,* can be used as reliable genetic markers for conducting molecular epidemiological studies. *Pvmsp1* gene is a mosaic organization of several variable blocks and its genotyping is based on detection of allelic variants in its three polymorphic fragments (F1 to F3).*Pvcsp* genotyping is based on detection of either of the two types of nonapeptide repeat units in its central domain; GDRA (A/D) GPQA, namely VK 210 type and ANGA (G/D) (N/D) QPG, namely VK 247 types.To determine allelic variants of *Pvcsp* and *pvmsp1*,a descriptive study was done on two-hundred and thirty blood samples of *P. vivax* collected from Sind and Baluchistan during 2008-2009.*Pvcsp* and *pvmsp1*were amplified using nested PCR methodology.PCR-RFLP was performed for genotyping of *Pvcsp* while different allelic forms of *Pvmsp1* were detected by analysis of fragment

size.Overall number of genotypes and their prevalence were defined arbitrarily by binning 20 base-pair (bp) intervals together. For *Pvmsp1,* it was found that in F1 fragment, 12 allelic variants were observed (bp size variation350-550), in *F2* fragment 17 allelic variants were observed (950-1270 bp) and in *F3* fragment 8 allelic variants were observed (250-390 bp).Thus, a total of 17 genotypes corresponding to *pvmsp1 gene* were found circulating in Southern Pakistan. *Pvcsp* genotyping in Pakistani isolates showed that *VK210* variants were predominant(79%, 182/230) while percent positivity of *VK 247* was 13%(29/230).Respective bp size variation were 600-870bp for VK 210 and 650-820bp for VK 247.We conclude that this is,to our knowledge, the first study from Southern Pakistan on genetic diversity in *pvcs* and *pvmsp1* gene. Data from this study indicates that both *pvcs and pvmsp1* can be used as reliable markers for conducting genotyping of *P. vivax.*Thus, this study may serve as a baseline data for future research on *P. vivax* diversity from Pakistan.

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PLASMODIUM VIVAX INFECTION IN DUFFY-NEGATIVE INDIVIDUALS IN ETHIOPIA: INDICATIONS AGAINST AN OLD PARADIGM

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In Ethiopia, the most wide-spread *Plasmodium* species co-exist and individuals of variant Duffy blood groups live in this country. A study was conducted to measure the prevalence of malaria and polymorphism in the *Duffy Antigen Receptor for Chemokine* (*DARC)* gene in East and South-Western Ethiopia. Presence of malaria was measured by microscopy and PCR. The polymorphism of *DARC* was analyzed by DNA sequencing. In this study, either *P. falciparum* or *P. vivax* infection was detected in all examined samples. In the analysis of the Duffy blood group, there were 17 (20%) and 24 (22.4%) homozygous Duffy negative individuals in Harar and Jimma study sites. Surprisingly, the data showed that *P. vivax* infection also occurred in three Duffy negative individuals. FYB/FYB^{null} was found to be the dominant genotype in both area. The FYA/FYB and FYB/FYB genotype was associated with susceptibility and FYB^{null}/FYB^{null} genotype was associated with protection against *P. vivax* infection. This study documents an emergence of *P. vivax* infection in Duffy negative individuals in the study area. Duffy negative blood group does not provide absolute protection of *P. vivax* infection in the studied population.

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CHANGING TRANSMISSION PATTERN OF *PLASMODIUM VIVAX* MALARIA IN THE REPUBLIC OF KOREA: RELATIONSHIP WITH CLIMATE CHANGE

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The Korean peninsula is an only place where indigenous *Plasmodium vivax* malaria has occurred continuously on a large scale in temperate areas. *P. vivax* malaria was endemic on the Korean peninsula for many centuries until the Republic of Korea (ROK; South Korea) was declared as a "malaria elimination" country in 1979. *P. vivax* malaria re-emerged in 1993 in the ROK, and has occurred constantly for more than 15 years after its re-emergence. *P. vivax* malaria in ROK has been strongly influenced by infected mosquitoes originating from the Democratic People's Republic of Korea (North Korea). Korean *P. vivax* malaria has shown typical characteristics of unstable malaria transmitted only during the summer season, and displays short and long incubation periods. The changing pattern of the transmission period can be predicted by analyzing the seasonal characteristics of early primary attack cases with a short incubation period. Such cases began to gradually occur earlier in the 1990s after the re-emergence. Considering the sporogony cycle in *Anopheles* mosquito and the asexual cycle via a short incubation period in the infected human, the period of transmission from malaria patients (via the vector mosquito) to manifestation as early primary attack cases would be 3-4 weeks. If *P. vivax* malaria is transmitted actively, subsequent minor peaks are expected to be seen after the annual highest peak by 3-4 week intervals. The obvious minor peak had not been seen until the early 2000s, however, it began to be seen in the mid-2000s. During 2006-2009, three or four subsequent minor peaks after the highest peak were observed by 20-day or 30-day intervals. This phenomenon shows that the length of transmission period of *P. vivax* malaria in the ROK has been gradually extending. This result may be ascribed to a climate change-mediated temperature rise. Malaria and climate data should be integrated to analyze and predict the influence of climate change on malaria occurrence in ROK.

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MORTALITY ATTRIBUTABLE TO *PLASMODIUM VIVAX* MALARIA

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Plasmodium vivax causes almost half of all malaria cases in Asia. Although once regarded as benign, studies have highlighted its association with severe and fatal malaria. The extent to which *P. vivax* contributes to mortality in endemic regions is not known. We aimed to define the epidemiology of mortality attributable to *vivax* malaria in southern Papua, Indonesia by conducting a retrospective clinical records-based audit of all deaths in patients with *vivax* malaria at Mitra Masyarakat Hospital. Between January 2004 and September 2009, hospital surveillance identified 3,495 inpatients with *P. vivax* monoinfection and 65 (1.9%) patients who subsequently died. Charts for 54 of these 65 patients could be reviewed, 40 of whom had pure *P. vivax* infections on cross-checking. Using pre-defined conservative criteria, *vivax* malaria was the primary cause of death in 5 cases, a major contributor in 17 cases and a minor contributor in a further 13 cases. Extreme anemia was the most common primary cause of death for patients in the first category. Malnutrition, sepsis with respiratory and gastrointestinal manifestations, and chronic diseases such as HIV infection were the commonest attributed causes of death for patients in the latter two categories. There were ~293,763 cases of pure *P. vivax* infection in the community during the study period giving an overall minimum case fatality of 0.12 per 1,000 infections. The corresponding case fatality in hospitalized patients was 10.0 per 1,000 infections. Although uncommonly directly fatal, *vivax* malaria is an important indirect cause of death in patients with malnutrition, sepsis syndromes and chronic diseases in southern Papua.

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CYTOKINE DYNAMICS AFFECT SUSCEPTIBILITY TO *PLASMODIUM VIVAX* AND *P. FALCIPARUM* INFECTION AND ANEMIA: STUDIES FROM THE PERUVIAN AMAZON

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The dynamics of immune factors in *Plasmodium falciparum* and *P. vivax* infection are thought to dictate host symptoms and pathology but have not been fully elucidated to date. We aimed to describe the cytokine dynamics of malaria infection and examine malaria species, age and gender dependent differences. Serum samples were collected by active

weekly blood sampling in the Zungarococha community of Iquitos, Peru from April 2003-September 2008 from a total of 397 *P. falciparum*, 515 *P. vivax* and 39 mixed *P. falciparum* and *P. vivax* infections. Cytokine measurements were made using Luminex bead-based assay from sera collected from infected individuals one week before, during, one week after and one month following infection. Relationships of immune markers to strain, age, and gender were assessed. *P. falciparum* and *P. vivax* infected males were more prone to febrile malaria infection and demonstrated higher levels of IFN-γ, TNF-α and IL-10 than infected females ($p<0.05$). A comparison of cytokine dynamics between the two strains revealed increased IL-4 and IL-6 levels in *P. falciparum* infected individuals one week following treatment ($p<0.05$). Moreover, IL-10 and IFN-γ levels were markedly decreased one week following treatment of *P. falciparum,* differing from the sustained elevation in these cytokines observed a week following treatment of *P. vivax* infection ($p<0.05$). TNF receptor and IL-10 levels were predictive of febrile infection and parasite density in both *P. falciparum* and *P. vivax* infection ($P<0.005$). Independent of parasite density, for both malaria species, hematocrit levels were directly correlated to IL-1 ($P<0.01$) while erythropoietin levels directly correlated with TNF-α ($P=0.003$). Interestingly, both hematocrit ($P=0.02$) and erythropoietin ($P=0.03$), were negatively correlated with the co-presence of IL-1 and TNF-α. There was a gender and species related difference in the hematocrit dynamics over time. In conclusion, these findings are the first to describe cytokine dynamics of malarial infection *in vivo* and demonstrate a strong association of inflammatory responses with parasite density, the severity of symptoms, and anemia. The observed differences in immune responses between genders and malaria species may explain distinctions in clinical presentations between males and females as well as core differences in the pathogenesis of *P. vivax* and *P. falciparum* infection.

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IMMUNE RESPONSE TO *PLASMODIUM VIVAX* INFECTION: A STUDY IN THE CENTRAL CHINA

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Plasmodium vivax infection possesses a characteristic of relapsing fever indicating the re-infection by previously hidden parasites in the host. The relapsed infection can lead to activation of memory T cells pool which might bring up protective immunity. This study aims to characterize natural immune responses in acute *P. vivax* infected patients living in a sole *P. vivax* infection cohort in Central China. Lymphocytes were collected from three recruitments: patients infected with *P. vivax*, malaria-immune and malaria-naive controls. Using flow cytometry, we showed memory T cells were elevated in blood during acute infection. The level of γδ T cells was two fold higher than that of naïve controls. This suggested that two populations, memory and γδ T cells, responded specifically to the *P. vivax* parasites. On contrary, B, NK and NKT cells were decreased during acute infection. In addition, regulatory T cells were reduced, suggesting the non-immune suppressive role of *P. vivax* parasites. Interestingly, *P. falciparum* antigens cross-stimulated T cells obtained from these *P. vivax*-infected patients. These results provided a further insight into interaction between *P. vivax* parasites and host cell-mediated immunity in the exclusive *P. vivax* endemic area that could be important for future development of a successful vaccine designation.

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EVALUATION OF NATURALLY ACQUIRED HUMORAL IMMUNE RESPONSES AGAINST IMMUNOREACTIVE PROTEINS OF *PLASMODIUM VIVAX* BY PROTEIN ARRAYS

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In the previous report, we successfully applied an antibody-based protein array for immunoprofiling of *Plasmodium vivax* infection, and some highly immunoreactive proteins were identified. To further characterize the antibody reactivity of these immunogenic proteins, we used a Ni-NTA surface based protein array to detect the immune responses from sera of *vivax* malaria parasites. ETRAMP, Pv12, Pv41 and MSP3.9 were studied to compare with the well-characterized *vivax* vaccine candidate MSP1-19. Among the 52 microscopically positive samples, all samples were detected *P. vivax* by the PvMSP1-19 arrays (100% sensitivity), and were 36 (69.2%), 31 (59.6%), 23 (44.2%) and 22 (42.3%) by ETRAMP, Pv12, Pv41 and MSP3.9 arrays respectively. The false positives were obtained 2 (95.0% specificity), 2 (95.0%), 3 (92.5%), 4 (90.0%) and 4 (90.0%) among 40 sera samples from healthy subjects. The ratio (fluorescent intensity of positive samples/that of negative samples) of antibody response to the PvMSP1-19, ETRAMP, Pv12, Pv41 and MSP3.9 were 17.5, 6.1, 2.6, 3.5 and 4.4 respectively. Although the naturally acquired humoral immune responses against PvMSP1-19 show superior reactivity than others, these recombinant proteins were also highly recognized by *P. vivax* infected patient sera. These results validate the protein arrays for profiling antibody responses to *P. vivax* infection, and further confirmed that ETRAMP, Pv12, Pv41 and MSP3.9 could also be selected as potential target antigens for malaria vaccine.

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CHARACTERIZATION AND SEROLOGIC RESPONSES TO *PLASMODIUM VIVAX* DUFFY BINDING PROTEIN (DBP) VARIANTS IN RESIDENTS OF PURSAT PROVINCE, CAMBODIA

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The *Plasmodium vivax* Duffy Binding Protein (DBP) is the ligand in the major pathway for *P. vivax* invasion of human reticulocytes, making it an appealing vaccine candidate. Region II of DBP (DBPII) is the minimal portion of the ligand that mediates recognition of the Duffy Antigen Receptor for Chemokines (DARC) on the reticulocyte surface and constitutes the primary vaccine target. Analysis of natural variation in the coding sequences of DBPII revealed signature evidence for selective pressure driving variation in the residues of the putative receptor-binding site. We hypothesize that anti-DBP immunity in *P. vivax* infections is strain-specific and hindered by polymorphic residues altering sensitivity to immune antibody inhibition. To comprehend the human IgG response following *P. vivax* infections we investigated the specificity of serum IgG in residents living in Pursat Province, Cambodia. Using ELISAs, we quantified the antibody titer against five variant alleles of DBPII. We also sequenced the DBPII of the field isolates to determine their relationship to the variant alleles used in the ELISAs. When correlating the IgG titer between the DBP

variants a strain-specific immune response was observed in patients with a high antibody titer to DBPII_AH as compared to the other variants. This differed from the correlation of high antibody titers between DBPII_P and DBPII_7.18 (ρ=0.88, p-value<0.0001) and DBPII_P and DBPII_O (ρ=0.87, p-value<0.0001). There appeared to be little correlation between specific polymorphic residues and IgG titer. Understanding the immune response to the polymorphisms within DBPII will allow further identification of epitopes to enable the production of a more effective *P. vivax* vaccine.

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NOVEL HUMANIZED MOUSE MODELS FOR *PLASMODIUM VIVAX*

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Development of humanized animal models able to sustain infection with *Plasmodium vivax* is required to increase our understanding of the biology and pathogenesis of the parasite and to test vaccines and anti-malarial drugs. Such models are also expected to allow development of a convenient mosquito challenge model for vaccination trials. We have developed novel humanized mouse strains that were genetically modified to develop a human hematopoietic system upon infusion of stem cells from umbilical cord blood. The humanized mice are immunodeficient, as they are knocked out for Rag and IL2 genes, and at the same time express human HLA-DR4. These mice develop human erythrocytes and reticulocytes and our preliminary data indicated their ability to sustain *P. vivax* blood stage infection. The ability of humanized mice to develop human T cells (both CD4+ and CD8+ subsets), B cells, and significant serum levels of human IgM and IgG, enable them as a unique model to test the immunogenicity and protective efficacy of human vaccines.

1540

FAILURE TO INFECT: DELINEATING *PLASMODIUM VIVAX* DEVELOPMENT CESSATION AMONGST *ANOPHELES DARLINGI* IN THE PERUVIAN AMAZON

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Malaria continues to be a top infectious disease burden in tropical and subtropical areas of the world. *Plasmodium vivax* is geographically the most widely distributed cause of malaria, with up to 2.5 billion people at risk and an estimated 80 million to 300 million clinical cases every year. Recent disease control innovations have included attempts at transmission blocking vaccines (TBVs), which target the intra-mosquito part of the complex malaria life cycle. Naturally occurring transmission blocking has been observed such as in a study conducted in the Peruvian Amazon in which mosquitoes dissected seven days after parasitemic blood meals revealed infection rates of only 50%. To further understand this pattern of incomplete transmission, we are performing membrane-feeding assays in which *vivax*-infected blood samples from subjects enrolled in the Peruvian Amazon are fed to first generation lab-reared mosquitoes. Instead of dissecting at day 7 as typically done to assess for oocysts, we are dissecting mosquitoes at multiple time intervals to detect more specifically where transmission drops - is it the failure of microgametes to fertilize macrogametes, failure of zygotes to transform into ookinetes or failure of ookinetes to traverse the midgut epithelium and become oocysts. To account for all developmental stages, dissections begin at 15 minutes post feed, up to 24 hours, then again at days 4 and 14. Blood samples are then stained with Giemsa and examined under microscopy. Of the 6 subjects already enrolled, we have found that overall parasitemia was important to transmission, but that it did not explain the lack of transmission in some subjects. Over the next several weeks, we will enroll a total of 20 patients as the malaria season in Iquitos is now at its busiest. By identifying termination points in sporogonic development, we aim to provide further insight into the biologic mechanisms pertinent to transmission blocking vaccine strategies and hope the results will help focus our search for transmission blocking antibodies in simultaneously collected plasma.

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GENETIC VARIABILITY OF *PLASMODIUM VIVAX* IN THE NORTH COAST OF PERU AND THE ECUADORIAN AMAZON

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Plasmodium vivax is the most widespread malaria parasite causing significant morbidity worldwide. In the Peruvian North Coast (PNC), the number of *P. vivax* malaria cases has steadily increased over the last few years despite a significant decline in the number of cases in Peru. To understand the transmission dynamics of *P. vivax* populations between the PNC and the neighboring Ecuadorian Amazon (EA), we studied the genetic diversity and population structure of *P. vivax* isolated in those areas. One hundred and twenty blood or serum samples comprising 95 PNC (58 from Piura and 37 from Tumbes, collected from 2008 to 2010) and 25 EA (from Puyo, collected between 2001 and 2004) were assessed by 6 polymorphic neutral microsatellite markers. Genetic variability was determined by the haplotype frequency and expected heterozygosity (He). Population structure was assessed by Bayesian inference cluster analysis. We found very low genetic diversity in PNC, with a single allele per locus, one haplotype and He=0 in Piura; and 1-3 alleles per locus, 3 haplotypes and He=0.30-0.32 in Tumbes. In contrast, high genetic diversity was observed in EA, with 4-6 alleles per locus, 15 different haplotypes and He=0.43-0.70. Population structure analysis revealed three distinct populations correlating with each geographic location. Five out 37 (14%) isolates from Tumbes had an identical haplotype to that found in Piura, suggesting unidirectional gene flow from Piura to Tumbes (100 Km apart). In addition, one haplotype collected in 2008 in Tumbes showed high similarity to a haplotype found in 2003 in Puyo, which was likely an imported case from the Ecuadorian Amazon. No shared haplotypes between Piura and Puyo was observed (300 Km apart), indicating little gene flow between these two areas. Our study provides important information on the transmission patterns between the Coastal areas of Peru and the Ecuadorian Amazon. Future studies should include isolates from the South Coastal region of Ecuador in order to identify other routes of parasite dissemination between the North Coast of Peru and Ecuador.

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SIMILAR DIVERSITY OF THE MEROZOITE SURFACE PROTEIN 3 ALPHA (MSP-3α) SUBFAMILY IN THAI AND VENEZUELAN *PLASMODIUM VIVAX* POPULATIONS

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Characterizing the polymorphism and identifying possible signatures of selection continues to be an important task in the study of malarial parasite antigens. This is of particular importance with regard to *Plasmodium vivax* blood stage antigens, which have not been as extensively studied as those from the more virulent *P. falciparum*. Of particular significance is the merozoite surface protein 3 (MSP-3) gene family, involved in the invasion of the asexual merozoite parasite form into the red blood cell. The MSP-3 gene family has undergone an expansion in the *P. vivax* lineage with twelve identified genes, compared to the four

in *P. falciparum* and two in *P. knowlesi*. This duplication event has been posed as being biologically important and could have implications for pathogenicity and parasite interactions with the host immune system. Here we describe the genetic diversity and identify possible signatures of selection of three paralogous and syntenic genes of the subfamily MSP-3α (2,559-2,724 base pairs in length), from Venezuelan and Thai population samples. Two of these paralogous genes have only recently been recognized. Despite differences in transmission rates, similar patterns of selection and polymorphism were found for the Thailand and Venezuelan samples. Genetic diversity, calculated by the parameter ϖ, revealed that the majority of sequence diversity was constrained to the N terminal for all three paralogs. This pattern holds when the two populations are compared. In contrast, strong evidence of positive selection was observed for the N-terminal in the two newly recognized MSP-3α gene copies, but only in the Thai population. Finally, evidence of purifying selection at the C-terminal for all three genes suggests that it could be of functional importance. If such functional constrain is demonstrated, it may be possible that the MSP-3 family is functionally redundant, a possible benefit to the parasite that may be conferred by increased antigenic diversity allowing enhanced evasion of the host immune response.

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EPIDEMIC AND INTEREPIDEMIC PERIODS STRUCTURE *PLASMODIUM VIVAX* POPULATION CIRCULATING IN FRENCH GUIANA BETWEEN 2006 AND 2010

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Since 2005, *Plasmodium vivax* is the main species circulating in French Guiana with an incidence of 51% in 2010. Understanding the genetic structure of this parasite population is essential in order to predict the rapid spread of certain phenotypes of interest (eg resistant parasites) or for a better understanding of the epidemiology. In this study, 195 isolates, collected between 2006 and 2010, in five geographical regions of French Guiana, were analyzed using six highly polymorphic microsatellite markers amplified by a semi-nested polymerase chain reaction method. We have shown that 28.1% of infections were polyclonal. The parasite population circulating in this department is likely drawn from a single ancestral population. The current population was genetically diverse (He = 0.68), and not structurated in time or space, as shown considering unique haplotype sampling strategy. In contrast, using all data samples, the population presents higher structuration rates, likely related to epidemics, which induce locally, and temporary high levels of inbreeding. Demographic history was also investigated, using both exponential and linear expansion models. Exponential model would consider that expansion of efficient population size occur mainly during epidemics, via clonal expansions. In contrast, linear model relies on genetic diversity acquired during sexual stages of *Plasmodium*. Exponential model shows a stable efficient population size, suggesting that epidemics do not contribute to long-term *Plasmodium* expansion. In contrast, linear model shows an expansion of efficient population size since some decades: this is related to a global increase of malaria cases, even when epidemic events are not considered. We conclude that epidemics may promptly and locally drive genetic information, but do not contribute to a wider, longer-term and regionally-scaled demographic history of *P. vivax*. Therefore, a similar study encompassing a larger geographical area would be required for a better understanding of the structure population of *P. vivax* in the Guiana Shield or even, Amazonia, and to confirm that *P. vivax* diversity is not proportional to the transmission level as observed with *P. falciparum*.

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LIMITED GENETIC DIVERSITY AND CLONAL POPULATION STRUCTURE OF *PLASMODIUM VIVAX* PARASITES FROM A GEOGRAPHICALLY ISOLATED COMMUNITY IN THE PERUVIAN AMAZON

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Plasmodium vivax has the highest burden of malaria morbidity and is the most spread human plasmodium species around the world. However, due to its complex life cycle, little is known about the genetic characteristics of these parasites populations and the epidemiology of the disease. In the present study, we explore the genetic diversity and population structure and dynamics of *P.vivax* parasites from a community in the Peruvian Amazon within the framework of a two years cohort looking for some insights that may explain the epidemiology of this disease within this area. Thirty-eight patients from San Carlos community, a geographically isolated area, were enrolled and followed up for 2 years after received the radical cure treatment (chloroquine + primaquine). *P. vivax* infections were detected by microscopy and by specie specific PCR. Molecular genotyping of *P.vivax* parasites using 15 microsatellites was performed. The genetic diversity was determined by calculating the expected heterozygosity (He) and allelic richness. The genetic population structure was determined calculating the linkage disequilibrium (pairwise LD and I_A^S), the probability of admixture or clonal reproduction (Psex) and looking for clusters of genetically related haplotypes. A limited genetic diversity of *P. vivax* parasites (He 0.47) and a high prevalence of monoclonal infections (83%) were found. The strong linkage disequilibrium (pairwise LD $P<10^{-6}$ and I_A^S 0.51), the low probability for admixture (Psex<0.0007) and the finding of a few clusters of genetically related haplotypes, described a clonal population of malaria parasites. The limited genetic diversity and clonal structure of *P. vivax* population described in this area may represent a high risk for the rise and spread of drug resistance. Nevertheless, also may stimulate positively the development of clinical immunity by the people in the Amazon Basin being reflected in the high proportion of asymptomatic cases reported in this community.

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CPG-DNA ENCAPSULATED WITH PEPTIDE ANTIGENS OF *PLASMODIUM VIVAX* IN MICROPARTICLES ENHANCES THE SYSTEMIC AND MUCOSAL IMMUNE RESPONSES IN MICE USING INTRANASAL MODE OF DELIVERY: AN APPROACH TOWARDS MUCOSAL VACCINE FOR MALARIA

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Due to drug resistance and limitation of growing of *Plasmodium vivax* parasite *in vitro* for enough DNA/Protein, we attempted an alternate synthetic peptide approach from the deduced amino acid sequences of different antigens of *P. vivax* constituting all the stages of the life cycle. For producing efficient and long lasting humoral immune responses, PLGA microparticles were used as delivery vehicles and CpG ODN as immunoadjuvants.The adjuvants used were synthetic oligonucleotides containing CpG (CpG-ODN 1826 and 2006, class B) motifs. *P. vivax* peptides viz, MSP 1#1, MSP 1#23, CSP, AMA, and Pvs24 (TBA) possessing B and T cell epitopes were synthesized using Fmoc chemistry, purified to homogeneity by Gel permeation chromatography and HPLC. Peptide purity was confirmed by amino acid acid analysis. These peptide

antigens were then entrapped in microparticles along with CpG-ODN. Biodegradable microparticles were prepared from 50:50 PLGA by water-in-oil-in water (w/o/w) solvent evaporation method. Particle size and size distribution was determined using particle size analyzer.The morphology was studied by scanning electron microscopy. Outbred strains of mice were immunized using intranasal route with different peptide formulations. Peptide specific IgG,IgA and SIgA estimation was done by standardized ELISA protocol. Peptides were found to be > 95% pure. Percentage peptide entrapment was in the range of 60-70%. Percentage entrapment for CpG-ODN was in the range of 50-60%. Microparticles were in the size range of 2-5µm and spherical with smooth surface. Presence of CpG in microparticles along with the peptide antigens showed serum IgG titre of 51,200-204,800 maintained till 90 days post immunization.The isotypic profile of the serum IgG revealed IgG2a/2b as the predominant isotypes,maintained till 90 days post immunization. Peptide specific IgA titre in sera ranged between 12,800-25,600 maintained till 90 days post immunization and SIgA titre in washes ranged between 800-25,600.Infected mosquitoes fed with high titer Pvs24 (TBA) anti-sera showed signinificant reduction in the oocyst count as revealed by membrane feeding assay.This study shows CpG ODN to be a potent mucosal adjuvant to induce immune responses against peptide antigens administered by intranasal inhalation.This is the first reported study with mucosal vaccination for malaria.

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FINE-SPECIFICITY OF HUMORAL IMMUNE RESPONSES GENERATED IN NAIVE ADULTS FOLLOWING VACCINATION WITH VMP001, A PREERYTHROCYTIC VACCINE CANDIDATE BASED ON THE CIRCUMSPOROZOITE PROTEIN OF *PLASMODIUM VIVAX*, FORMULATED WITH GSK BIOLOGICALS ADJUVANT SYSTEM AS01$_B$

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We have previously reported on the development of VMP001, a chimeric circumsporozoite protein based vaccine for *P. vivax*. In previous studies the VMP001 vaccine candidate demonstrated strong immunogenicity, and also demonstrated high efficacy in *Aotus* monkeys challenged with infectious *P. vivax* sporozoites. Based on these data we proceeded to test VMP001 for its safety, immunogenicity and efficacy in humans. This first-in-human study was designed as a dose-escalating study using three doses of VMP001 formulated in GSK Biologicals Adjuvant System AS01$_B$.The primary study endpoint was to assess vaccine safety. One of the secondary endpoints of this study was an evaluation of the fine-specificity of humoral immune responses generated following vaccination. We evaluated the antibody responses from volunteers to the N- and C-terminal regions as well as the central repeat region of the synthetic VMP001 vaccine construct. The vaccine was immunogenic and all three doses of vaccine induced antibody responses that spanned the entire length of the vaccine construct. While there were detectable responses to all domains of the molecule, there was a distinct hierarchy in terms of the regions recognized by the vaccinees. The C-terminal region induced the highest magnitude of responses in all three groups, followed by the N-terminal region with the Repeat domain the lowest. While the magnitude of the titers differed, in general, titers against any single domain correlated to titers against the others. Individuals having the highest antibody titer to one component of the vaccine also had high titers to the other domains. Although the vaccine did not induce sterile protection, a small but consistent delay in prepatent period was observed in some subjects. Detailed analysis of the immune response may help understand these findings and help improve the design of future vaccine constructs. We will discuss vaccine induced antibody avidity and fine-specificity in detail.

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PLASMODIUM VIVAX CIRCUMSPOROZOITE PROTEIN-SPECIFIC CELLULAR IMMUNE RESPONSES AFTER IMMUNIZATION WITH THE VMP001/AS01$_B$ CANDIDATE MALARIA VACCINE IN MALARIA-NAÏVE INDIVIDUALS

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Plasmodium vivax is the major cause of malaria outside of sub-Saharan Africa and inflicts debilitating morbidity and consequent economic impact in developing countries. We have developed a novel chimeric recombinant protein VMP001 based on the CSP of *P. vivax*. The first-in-humans safety, immunogenicity and efficacy clinical trial of VMP001 formulated in GSK Biologicals' adjuvant system AS01$_B$ was recently performed in malaria-naïve adults. An effective *P. vivax* vaccine will likely require induction of both humoral and T cell responses. The aim of this study was to measure vaccine-induced T cell responses in PBMCs collected at various time points following immunization. Multiparameter flow cytometry was used to enumerate the frequency and phenotype of T cells producing IL-2, TNF, and IFN-γ after *in vitro* stimulation of cryopreserved PBMCs with VMP001, or pools of overlapping peptides corresponding to different regions of the VMP001 construct. IL-2$^+$CD4$^+$ T cell responses were detected in all volunteers at one or more time point. TNF$^+$CD4$^+$ T cell responses were detected in 93% of volunteers, albeit at a lower frequency than IL-2$^+$CD4$^+$ T cells. In addition, IFN-γ$^+$CD4$^+$ T cell responses were detected in 55% of volunteers. TNF$^+$IL-2$^+$ double-producers and IL-2$^+$ single-producers were most commonly detected, along with a smaller population of cells producing all three cytokines tested. The vaccine-induced CD4$^+$ T cell responses were strongest and most frequently detected against the N-term region (90% of volunteers) but smaller responses against C-term and repeat regions were also observed (24% and 21% of volunteers respectively). Our results indicate that the VMP001/AS01$_B$ vaccine was immunogenic, as indicated by the detection of antigen-specific CD4$^+$ T cell responses in all immunized volunteers. Although the vaccine did not induce sterile protection, a small but consistent delay in prepatent period was observed in some subjects. Detailed analysis of the induced T cell responses may help understand these findings and help improve the design of future vaccine constructs.

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A ROBUST *PLASMODIUM VIVAX EX VIVO* INVASION ASSAY

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We describe a protocol for an *ex vivo Plasmodium vivax* invasion assay that can be easily deployed in laboratories located in endemic countries. The assay involves mixing enriched cord blood reticulocytes with matured, trypsin-treated *P. vivax* schizonts concentrated from clinical isolates. The invasion efficiencies observed for parasites from 85 isolates were highly variable, ranging from 0.1% to 22.3% with a mean of 3.7% (95%CI: 2.8%-4.6%). The utility of the protocol for vaccine testing

was demonstrated by using it as an invasion-inhibition assay to assess functionally antibodies against DARC, pvMSP1 and pvDBP. This provides the first biological demonstration that polymorphisms in the pvDBP gene affect the invasion inhibition efficacy of anti-pvDBP antibody.

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IS THAT A RODENT IN YOUR LUGGAGE? BUSHMEAT CONFISCATIONS REPORTED IN THE CENTERS FOR DISEASE CONTROL AND PREVENTION'S QUARANTINE ACTIVITY REPORTING SYSTEM - UNITED STATES, SEPTEMBER 2005-DECEMBER 2010

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Bushmeat, defined as raw or processed meat derived from wild animals, is considered a potential source of infection. The HIV epidemic has been associated with the hunting and processing of bushmeat, and recent studies have found evidence of simian foamy viruses in bushmeat samples confiscated at United States ports of entry. Existing US regulations prohibit importation of bushmeat from specific animals. However, illegal importation still occurs, and the exact amount of imported bushmeat is unknown. This project describes bushmeat confiscation reports in the Centers for Disease Control and Prevention (CDC) Quarantine Activity Reporting System (QARS) and attempts to identify geographic and seasonal trends. A keyword search was performed in QARS to capture all bushmeat-related reports from September 2005 through December 2010. All relevant reports were reviewed and compiled in an analytic database. All items were categorized by CDC-regulated species, including nonhuman primate, rodent, bat, bird, unknown, and other. In total, 543 confiscated bushmeat items, weighing 2303.6 kilograms (kg), were reported and recorded in QARS. The median weight of bushmeat per report was 2 kg and ranged from 0.1 to 650 kg. Half of confiscated bushmeat was identified as rodent. The most confiscations were reported in 2008 and the least in 2006. Africa was the most frequent continent of origin, with 68% of all confiscated bushmeat originating from Ghana and Nigeria. Seasonality was evident, with bushmeat confiscations peaking in late spring to early summer after adjustment for travel volume. Four times more bushmeat was confiscated during an enhanced surveillance program in June 2010 compared to the same period in previous years, suggesting that items were missed during routine inspections. Even with regulations in place, bushmeat is smuggled into the United States. Longstanding cultural practices make it difficult for persons to accept potential health risks. Therefore, enforcing penalties associated with bushmeat confiscations, along with health education aimed at high-risk groups, may be useful to deter import attempts.

1550

TEACHING GLOBAL HEALTH IN THE UNDERGRADUATE LIBERAL ARTS

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Teaching public health in the undergraduate curriculum of four-year institutions has moved forward over the last five years with the Educated Citizen and Public Health initiative. It has been driven by leaders in public health, the arts, sciences and humanities, and public health organizations, as well as by student interest. There has been equal interest in global health with a focus on health equity for all persons and particularly for those in low-income countries. Most undergraduate offerings in global or public health have been aligned with universities with graduate programs in the disciplines. However, is likely that strong interest in global health exists at all institutions including liberal arts colleges. Following the experience of teaching a short course in global health in a liberal arts college, student organizations, courses, and officially recognized curricular offerings in global and public health were identified for the 2009-2010 academic year for fifty of the top ranked liberal arts colleges in the United States. 42% of the colleges had a track, concentration or program; schools that did not have official themes still listed at least one course in global or public health. All of the themes were interdisciplinary and when they were more expansive such as a program, they were organized as a multi-college consortium. 48% of them had been developed since the 2005 academic year. The most number of courses were in the Social Sciences (n=9.9±12.2) followed by the Natural Sciences (3.2±5.3). Student organizations in global or public health were present on 30% of campuses and all but two schools had service, social justice or AIDS organizations. The values of a liberal education are closely aligned with those of global health: social responsibility, critical thinking, skills in communication, analysis and problem-solving, ethical reasoning, and knowledge of the wider world through integrated study in the arts and sciences. Liberal arts colleges can take several steps to capture this interest in global health and enhance their curriculum.

1551

GETTING THE NUMBERS RIGHT: CONSIDERATIONS FOR QUANTIFICATION OF MALARIA MEDICINES AND RAPID DIAGNOSTIC TESTS IN RESOURCE LIMITED SETTINGS

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Countries continue to scale up interventions for the treatment, diagnosis, and prevention of malaria. Central to the success of these interventions is ensuring that a consistent supply of products is available whenever and wherever they are needed. Quantification is the process of estimating the quantities and costs of the products required for a specific health program and determining when the products should be delivered to ensure an uninterrupted supply for the program. Quantification is a critical supply chain activity that links information on services and commodities from the facility level with program policies and plans at the national level, and is then used to inform higher level decision making on the financing and procurement of commodities. The results of a quantification can be used to help maximize the use of available resources for procurement, advocate for mobilization of additional resources when needed, and inform manufacturer production cycles and supplier shipment schedules. Malaria presents unique challenges to quantification due to: 1) changing epidemiology due to large scale implementation of effective malaria control interventions in diagnosis (particularly RDTs), net distribution campaigns, and increased use of ACTs; 2) seasonality and geographic considerations of disease; historical presumptive treatment of malaria with ACTs, rather than confirmed biological diagnosis; inaccurate records and weak reporting systems for malaria medicine consumption. The following recommendations are offered to help improve the accuracy of quantifications of malaria medicines: 1) in the short term, the effect of increased diagnosis and prevention efforts will likely not affect the quantities of ACTs required; 2) when developing a supply plan, program managers should arrange shipments to arrive prior to the peak periods of malaria, to avoid overstocking during the dry season, and understocking during the rainy season; 3) investments should be made in strengthening information systems so that future quantifications can be data driven rather than assumption driven; 4) forecasts should be made based on as many data sources (demographic/morbidity data, services data, and consumption data) as possible. The results of forecasts using different data sources should be analyzed and compared; and 5) quantifications should be updated on a quarterly basis with actual consumption data.

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URBAN POOR HARD HIT BY CHRONIC CONDITIONS

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Hypertension and diabetes are no longer diseases of affluence. High poverty levels in sub-Sahara Africa have heightened their prevalence.Poor people are suffering from these conditions because of rapid urbanization, cultural factors, poor health management and general effects of poverty. The study aimed at understanding diabetes and hypertension among urban poor, a total of 5,190 individuals were sampled. Data on their risk was indicated by tobacco use, alcohol consumption, diet and physical activity, 12 per cent of adult were current smokers and on average smoked eight cigarettes a day. 10 per cent were alcohol users and nearly a third 32 per cent were heavy drinkers. On physical activity, 15 per cent did not engage in any, while over half (54 per cent) had insufficient intake of fruits and vegetables, while 37 per cent had high salt intake. The prevalence rates among adult women were slightly higher than men. Increasing with age, respondents aged 40 and 60 likely having diabetes and 30 and 60 years having hypertension.Heavy alcohol use leads to damage of pancreases - organ producing insulin regulating how sugars are metabolized.A damaged pancreas results in less insulin therefore increasing levels circulating sugars (glucose specifically). "Diabetes is therefore a manifestation of imbalance in sugar metabolism.According to findings, some types of alcohol are a source of "empty calories," body gets a lot of calories without a feeling of satiety.In an ideal situation, one should get calories reflecting feeling of sanctification. More empty calories consumed, higher chances gaining too much weight, Interfering with sugar metabolism and increasing chances of diabetes, Leading damage inner lining of blood vessels which lead to high blood pressure. High consumption salt leads to high concentrations salt in blood, which body compensates for by absorbing more water from cells. Resulting an increased blood volume, putting pressure on kidneys and blood vessels, causing damage manifest as high blood pressure.

1553

PILOTING AND EVALUATING A GLOBAL CHILD HEALTH CURRICULUM FOR PEDIATRIC RESIDENCY PROGRAMS

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North American pediatricians care for a growing number of immigrant and refugee children, and training programs should reflect this changing demographic. A modular global child health curriculum designed for use during academic half-days was developed and piloted across four Canadian post-graduate training centers. Here we present the results of an evaluation of participant satisfaction and knowledge gain using a standardized satisfaction survey and pre/post multiple choice knowledge tests. 125 trainees participated from 4 pediatric training centres. 95% completed 2 or more modules and 42% completed all four. Scores on a standardized satisfaction questionnaire were internally consistent (Cronbach's alpha=0.88 to 0.95 for the four modules) and indicated a high level of participant satisfaction (mean (SD) satisfaction scores of 3.4 (0.6) to 3.6 (0.6) out of maximum total of 5). Analysis of determinants of participant satisfaction indicated that past participation in clinical electives abroad was associated with higher satisfaction scores. Scores on multiple choice knowledge tests increased following the teaching sessions ($p < 0.0001$). This finding remained after stratification by site, year of post-graduate training, and previous global health experience ($p<0.01$ for all comparisons), suggesting that participants in all subgroups demonstrated knowledge gain following participation in all four modules. Participants with lower pre-test scores demonstrated greater knowledge gain than those with higher pre-test scores ($p<0.0001$ for all modules), suggesting that participants with low baseline GCH knowledge benefited most from the modular curriculum. On the other hand, knowledge gain did not differ according to level of residency training or previous global health experience. Item analysis of the knowledge evaluation instrument demonstrated that 85% of questions were at an appropriate difficulty level, 35% discriminated well between top and bottom performers, 75% increased significantly after teaching sessions and 78% had a good item-to-total score correlation. In summary, satisfaction with the modular curriculum was high among participants, and knowledge gain could be demonstrated unequivocally. This standardized curriculum could be scaled up for implementation across North American pediatric training centers.

1554

CLINICAL TRIALS IN RESOURCE-LIMITED SETTINGS

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Clinical trials in developing countries lag far behind wealthier regions through a lack of knowledge and skills. More disease management trials are needed but there is a lack of access to generic clinical trial tools, guidance and training. Furthermore, researchers are daunted by regulations and guidelines. Existing capacity development activities are linked to specific trials and are disease focused; so limit diversification. Other factors such as the cost of travel and remote locations of potential research sites also restrict clinical trial capacity development in these regions. Global Health Clinical Trials (www.globalhealthtrials.org) is a new collaborative web-based platform. It is a free, open access and entirely collaborative platform where anyone working on trials can access guidance, tools resources and share their knowledge, views and experiences. This resource is evidence-led through integrated participatory action research that enables researchers based in these settings to identify the problems in their own context and contribute to solutions themselves. The platform was released as a pilot in May 2010 and within a year attracted over 1000 members from 56 developing countries. It is not for any one disease or just about product development trials. The aim is to support researchers in running their own trials and diversifying in the types of trial that they conduct. Researchers and their staff use the platform to seek expert and peer advice on diverse issues such as data management, intend-to-treat-analysis and setting up community advisory boards. There are free e-learning short courses to take researchers and research staff pragmatically through all the trial steps and processes. In partnership with WHO/TDR we have created an on-line continuing professional development scheme. This is the first of its kind and this free opportunity to build personal learning and training portfolios whilst guiding professional development will be highly impactful in creating a cadre of developing country clinical trialists. The Global Health Clinical Trials Programme is an open collaboration and is already gaining widespread recognition for being the single point of reference for accessing information, experts and support on all aspects of running clinical trials in resource-limited settings.

1555

USE OF BIOMETRIC DATA IN LINKING HEALTH DEMOGRAPHIC SURVEILLANCE SYSTEMS (HDSS) AND HEALTH MANAGEMENT INFORMATION SYSTEM (HMIS) INFORMATION IN IFAKARA AND RUFIJI HDSSS IN TANZANIA

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The objective of the study was provide a mechanism to link demographic and social economic information captured within HDSS frameworks and the health facility-based data. House to house fingerprints and facial images data collection was done for all HDSS members. Field workers each equipped with a net book computer, fingerprint scanner, web camera, methylated spirit (for cleaning fingers before fingerprint capture) visited households for 12 months from June 2010 up to May 2011. Field workers did a daily data backup and field supervisors did a weekly data integration. Once integrated the biometric data were taken to the surrounding health facilities within the Demographic Surveillance Area (DSA) where upon each visit a patient is checked using a combined search and data validation using fingerprints, facial image and the demographic data. Once identified the patient's visitation records including health services attendance, diagnosis, service and treatment data were captured and linked to his/her HDSS profile. In the two pilot health facilities in Rufiji HDSS from September 2010 to May 2011,726 patients have had their HDSS and HMIS information accurately linked using the deployed mechanism. At the moment essential data health system analysis, health planning and policy formulation is lacking because there is no such a system that links HDSS and HMIS information. Upon implementation of the exercise, they were concerns from the public on misuse of the biometric data collected and use of fingerprint scanner as a means for HIV/AIDS testing and for criminal tracking. Integrating the HDSS and HMIS data sources would provide both the numerator and the denominator population for computation of both disease incidence rates in the population and health service coverage rates in the health system.

1556

A REVIEW OF THE GEOGRAPHICAL VARIATION IN *PLASMODIUM VIVAX* RELAPSE RATE

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Plasmodium vivax has the widest global geographic distribution of the malaria parasites known to affect man. Contrary to past beliefs that it is a "benign" form of malaria, it has been shown to result in severe disease and mortality. Control of *P. vivax* is complicated by its ability to relapse after treatment of the initial infection. Hypnozoites, a dormant liver stage of infection, can initiate a relapse weeks or months following initial infection. The widely accepted epidemiological understanding is that strains of *P. vivax* from various geographical areas exhibit different relapse patterns, such that those found in tropical regions will relapse quickly (3-6 weeks) and those in temperate regions will relapse more slowly (6-12 months). Support for this belief is provided here, in a systematic review of published and unpublished reports of *P. vivax* relapse rates in patients not treated with primaquine, the only drug currently available to treat the hypnozoite stage of the infection. Statistical analysis was performed to identify the association between the rate of relapse and several climatic and geographical parameters. The relationship between relapse rate and environment was illustrated as a global map of 146 *P. vivax* relapse records plotted as georeferenced points over a mask of the longest unsuitability period (the length of time in months an area is unsuitable for malaria transmission). The regression model demonstrated an association between relapse rate and land surface temperature, precipitation, temperature suitability index (a measure of temperature conditions which allow for sporozoite development), and longest unsuitability period. The map of the median time to relapse, grouped into three classifications of relapse rate (≤60 days, 61-180, and >180 days), showed the occurrence of faster relapse (≤60 days) in regions nearly always suitable for malaria transmission, and slow relapse (>180 days) in regions only suitable for transmission four to six months of the year. While these results are based on a relatively small sample of data, given the limited information published on *vivax* relapse, the data, from more than 36,000 patients, offers insight into the poorly understood mechanism of relapse. Elimination efforts often leave *P. vivax* the last parasite standing, due to its ability to relapse. A better understanding of relapse therefore is an essential component to its control.

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PREVALENT PARASITEMIA, FEVER, ANEMIA AND INHERITED BLOOD DISORDERS IN MESO-ENDEMIC WEST SUMBA, INDONESIA: A RANDOMIZED, CROSS-SECTIONAL ANALYSIS

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The expected decline in the intensity of malaria in many areas across the globe in response to heightened malaria control efforts has placed increased emphasis on understanding low to moderate transmission settings such as that typified by Southeast Asia. We conducted a cross-sectional socio-demographic and parasitologic survey in a rarely studied region of West Sumba district, East Nusa Tenggara Province, Indonesia from August-November 2010. Blood smear analysis of 960 randomly selected individuals revealed meso-endemic malaria with a point prevalence estimate of 24.7%. *Plasmodium falciparum* infections predominated over *P. vivax*, with a few cases of *P. malariae*, and a single case of *P. ovale* noted. Age specific prevalence rates were highest in children and young adults less than 15 years of age, and geometric mean parasite densities were found to decrease with increasing age. The prevalence of fever (aural temperature ≥ 37.5 °C) among parasitized and non-parasitized study participants was 7.2% (17/237) and 5.3% (38/723) respectively, suggesting approximately 30% of fevers were likely to be associated with malaria infection. The inherited red blood cell (RBC) polymorphisms glucose-6-phosphate dehydrogenase (G6PD) deficiency and Southeast Asian ovalocytosis (SAO) were detected in 17.5% and 22.6% of study subjects respectively, but were not associated with the likelihood of parasitemia ($P > 0.2$) as detected by conventional light microscopy. The prevalence of anemia on the day of survey was 43.4% (403/928), and parasitemic study participants were more likely to be anemic than those without parasitemia (odds ratio [OR] = 1.46; 95% confidence interval [CI] = 1.08-1.96; $P = 0.014$). These findings are consistent with the few prior studies conducted in the region and indicate moderate malaria transmission in an endemic area of East Nusa Tengarra Province, that has been stable enough to induce some anti-parasite and anti-disease immunity. Although we did not observe an association between either SAO and G6PD deficiency and the likelihood of patent parasitemia, the impact of these polymorphisms on other aspects of infection such as sub-patent parasitemia, parasite density and severe clinical disease remains to be determined. The relatively small proportion of parasitemic study subjects with fever for the given transmission intensity in this study population merits further attention and will be a focal point of future studies.

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MORBIDITY AND MORTALITY CAUSED BY *FALCIPARUM* AND *VIVAX* MALARIA IN HYPO- TO MESO-ENDEMIC WEST SUMBA, INDONESIA: A TWO-YEAR RETROSPECTIVE HOSPITAL-BASED STUDY

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Plasmodium vivax causes what has often been referred to as benign tertian malaria. This study aimed to measure the relative contributions of *falciparum* and *vivax* malaria to the burdens of hospitalized, severe and fatal malaria the main referral hospital for West Sumba in eastern Indonesia. We systematically examined records of malaria screening and admission at Karitas Hospital (115 beds), which served a community of 280,000 people living with a median prevalence of about 6% of both *P. falciparum* and *P. vivax* (ranging from 1:1 to 2:1 in surveys). Conducted in 2010, we limited the survey to calendar years 2008 and 2009. Febrile patients seeking treatment at this hospital had routine and reliable microscopic blood film exams for malaria. Among 18,589 febrile patients screened, only 2711 were positive and managed as outpatients. Another 3484 found positive for malaria were admitted as inpatients and these records were screened for completeness, with 35 being excluded, leaving 3449 malaria patient records to evaluate. Relevant content of hospital records were manually transferred into 2 separate case record forms (CRF) per patient, which were then each double entered into an electronic database. Only fully reconciled CRFs were uploaded for analysis. Among the 3449 admissions for malaria, there were 614 patients classified as severely ill, and 66 patients did not survive. *Falciparum* malaria accounted for 65% and 70% of this morbidity and mortality, and *vivax* malaria contributed 32% and 27% to these burdens in this community. Whereas infants, children, and adolescents carried 82% of the morbidity and mortality burdens due to *P. falciparum* (OR=4.6; 3.2-5.8), they carried only 62% of this in *P. vivax* (OR=1.6; 1.2-2.3). Patients with *P. falciparum* were much more likely to be classified as having severe illness (OR=2.7; 2.2-3.3), but the risk of death with a classification of severe illness was the same between *falciparum* and *vivax* malarias (OR=1.2; 0.7-2.2). Anemia and cerebral syndromes dominated among the severely ill with both *falciparum* and *vivax* malaria (>90% of syndromes for each species). These findings show *P. vivax* contributed substantially to the burdens of morbidity and mortality in West Sumba and seemed to have provoked severe disease syndromes essentially identical to *falciparum* malaria in both character and risk of death.

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THE CHANGING EPIDEMIOLOGY OF MALARIA IN PAPUA NEW GUINEA

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Papua New Guinea (PNG) has the highest malaria transmission outside of sub-Saharan Africa. Moreover, the country's malaria epidemiology is more complex than many other places with four endemic malaria species and a variety of anopheline vectors filling the diverse ecological niches. Only recently, the National Department of Health has re-activated its malaria control program with the up-scaling of insecticide treated nets supported by a Global Fund to Fight AIDS, Tuberculosis and Malaria grant. A country-wide prevalence survey in 70 randomly selected villages investigated the malaria prevalence and species composition. Follow-up surveys in selected sites assessed changes after the introduction of mosquito nets. Malaria prevalence was assessed by light microscopy and PCR. A total of over 9000 blood samples were analysed by microscopy. Population parasite prevalence rates ranged from 0 to 57%, with *Plasmodium falciparum* prevalence between 0 and 29%, *P. vivax* between 0 and 27% and *P. falciparum* mixed infections between 0 and 9%. *P. vivax* infections dominated in 22% of the villages with detectable parasitaemia. Parasite prevalence decreased significantly with altitude, more so for *P. falciparum* than for *P. vivax*. 80% or higher mosquito net usage was independently correlated with lower parasite prevalence. Microscopy and PCR results are compared with historic data and discussed in the context of the large-scale roll out of mosquito nets, change in treatment policy, and observed changes in malaria transmission.

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THE FORCE OF INFECTION: THE KEY TO UNDERSTANDING THE EPIDEMIOLOGY OF *PLASMODIUM FALCIPARUM* MALARIA IN PAPUA NEW GUINEAN CHILDREN

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Genotyping *Plasmodium falciparum* parasites in longitudinal studies provides a robust approach for estimating force of infection (FOI) in the presence of superinfections. $_{mol}$FOI, defined as the number of new *P. falciparum* clones acquired over time, is molecular parameter equally suitable for describing basic malaria epidemiology as well as for measuring outcomes of clinical trials of antimalarial interventions. We investigated the potential of molecular parameters to explain differences in risk of *P. falciparum* infections and disease between wet and dry season, among different age groups and use versus non-use of insecticide treated bednets (ITN). 264 children 1 - 3 years of age from Papua New Guinea were followed over 16 months with active detection of infection at 2-monthly intervals and during episodes of febrile illness. PCR for the highly polymorphic genotyping marker merozoite surface antigen 2 was performed in all blood samples. To track individual parasite clones in consecutive blood samples with maximal resolution PCR fragments were sized by capillary electrophoresis. $_{mol}$FOI was identified as explanatory variable for precisely describing the risk of *P. falciparum* illness. $_{mol}$FOI was significantly correlated to incidence of episodes, irrespective of whether a parasite density cut off was applied or not. Seasonal variation was observed in $_{mol}$FOI, and thus in the risk of illness. $_{mol}$FOI was significantly higher during the rainy season than in the dry season. Our analyses suggest a central role of $_{mol}$FOI for explaining differences in the burden of clinical *P. falciparum* malaria in our cohort. $_{mol}$FOI almost completely explained spatial variation, age trends and effect of ITN use on incidence. Acquisition of new parasite clones seems to be a major factor for clinical illness in these children. This study highlights the suitability of a new parameter, $_{mol}$FOI, for understanding the epidemiology of clinical malaria in young children. We propose to apply the molecular determined parameter $_{mol}$FOI for monitoring effects of malaria interventions.

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PLASMODIUM VIVAX GAMETOCYTE DYNAMICS AND THE ROLE OF DRUGS IN REDUCING TRANSMISSION POTENTIAL

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Designing interventions that will reduce transmission of *vivax* malaria requires a detailed understanding of the dynamics of *Plasmodium vivax* gametocytemia. We analyzed data from a large randomized controlled trial in Northwestern Thailand and two trials in Papua, Indonesia to identify and compare risk factors for *P. vivax* gametocytemia at enrolment and during the 6 to 9 weeks following treatment. Overall 492 patients with *P. vivax* monoinfections were evaluable from Thailand and 476 patients with *P. vivax* infections (162 of whom had concurrent *P. falciparum* infections) were evaluable from Papua. In Thailand 84.3% (415/492) of patients with monoinfections were gametocytemic at enrolment (median gametocyte density = 266/µL) compared to 66.6% (209/314) in Papua (median gametocyte density = 113/µL; p<0.001 for gametocyte prevalence and density comparisons). At both sites there was a positive correlation between initial asexual parasitemia and gametocyte density (R=0.53 and R=0.47, p<0.001 for both). High asexual parasitemia was also associated with an increased risk of gametocytemia during follow-up. In Thailand, the cumulative incidence of gametocytemia between day 7 and 42 following dihydroartemisinin+piperaquine (DHA+PIP) was 6.92% versus 29.1% following chloroquine (p<0.001). In Papua, the cumulative incidence of gametocytemia between day 7 and 42 was 33.6% following artesunate+amodiaquine (AS+AQ), 7.42% following artemether+lumefantrine and 6.80% following dihydroartemisinin+piperaquine (p<0.001 for DHA+PIP versus AS+AQ and p=0.4 for DHA+PIP versus AM+LUM). Gametocytemia during follow up was associated with concurrent asexual parasitemia in 98.9% (172/174) of cases. *Plasmodium vivax* gametocyte carriage closely mirrors asexual stage infection. The most important strategy for interrupting *P. vivax* transmission is prevention of relapses, particularly in those with high asexual parasitaemia.

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WIDESPREAD INFECTION OF WILD-LIVING CHIMPANZEES AND GORILLAS WITH *PLASMODIUM VIVAX*-LIKE PARASITES

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Plasmodium vivax accounts for over 50% of malaria cases outside of Africa, but is not thought to be transmitted in western and central Africa because of the high prevalence of the Duffy-negative trait in local populations. Nonetheless, the finding that some individuals in west central Africa harbor antibodies to *P. vivax* surface proteins, together with reports of *P. vivax* in some travelers returning from this geographic region, have suggested that there might be an as yet undefined reservoir of *P. vivax*. Since we have recently found evidence of multiple species of *P. falciparum*-related parasites in apes, we used non-invasive methods to determine whether wild-living chimpanzee and gorilla populations are naturally infected with *P. vivax*. Using *P. vivax* specific primers to amplify a diagnostic (~300 bp) mitochondrial DNA (mtDNA) fragment, we screened 3,044 chimpanzee, 1,236 gorilla and 513 bonobo fecal samples from 80 different field sites. Although ape *P. vivax* was detected at an overall low frequency (~1-2%; possibly because of low fecal parasite loads), we found 45 chimpanzee and 30 gorilla samples from 30 field sites to harbor *P. vivax* sequences. Ape *P. vivax* was widely distributed among central (*P. t. troglodytes*) and eastern (*P. t. schweinfurthii)* chimpanzees, as well as western (*Gorilla gorilla gorilla*) and eastern (*G. beringei graueri*) lowland gorillas, but was absent from bonobos (*Pan paniscus*). Many of the *P. vivax* positive specimens also contained *Laverania* parasites, indicating that ape *P. vivax* occurs frequently in the context of mixed parasite infections. To confirm that the diagnostic PCR was specific for ape *P. vivax*, we used single genome amplification to generate larger mtDNA fragments (~3-4.5kb) from a subset of samples. Phylogenetic analyses of these sequences revealed that the ape parasites were nearly identical to each other as well as to human *P. vivax*. These findings document widespread infection of wild-living chimpanzees and gorillas with *P. vivax*-like parasites throughout central Africa.

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THE URINARY SCHISTOSOMIASIS-BACTERIURIA CONNECTION: A NEW MODEL TO EXPLORE POTENTIAL MECHANISMS

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Many studies have posited urinary schistosomiasis as a risk factor for bacterial urinary tract infections (UTI). Bacteriuria may worsen urinary schistosomiasis-linked morbidities such as dysuria and hematuria. One possible reason for reported high rates of *Schistosoma haematobium*-UTI co-infection is *S. haematobium* egg-induced shedding of bacteria-coated urothelial cells into urine, which could facilitate detection of UTI. Another possible mechanism is egg granuloma-related urinary tract obstruction, which could promote urinary stasis and bacterial growth. A third possible mechanism is a skewed immune response to *S. haematobium* eggs in the bladder which could preclude an effective local response to bacteria. Previously we established a mouse model of *S. haematobium* egg-induced, non-obstructive immunopathology. Injection of eggs into the anterior bladder wall avoids obstruction of the ureters and bladder outlet. We used this model to test the urothelial shedding and immune skewing hypotheses for the urinary schistosomiasis/UTI association. At 1 and 2 weeks post-injection, 30% and 36% of egg-injected mice featured urothelial shedding vs none of the vehicle-injected mice. When mice were transurethrally administered uropathogenic *E. coli* 7 days after egg injection, bacteriuria occurred at high rates (75%) and titers (median $8x10^5$ cfu/ml) vs vehicle-injected mice and mice infected with *E. coli* only (both groups 33% and median 0 cfu/ml). The bladders of egg- vs vehicle-injected mice featured more neutrophils (17.6%±4.25 vs 5.07%±1.09; p=0.0076) and eosinophils (1.62%±0.26 vs 0.799%±0.799; p=0.0151), suggesting a role for schistosome-induced immune skewing in susceptibility to bacteriuria. We are working to further dissect the mechanisms by which urothelial shedding and/or immune skewing may promote synchronous urinary schistosomiasis and bacteriuria. These studies will enhance understanding of how bacterial uropathogens may exploit host immune responses "distracted" by urinary schistosomiasis, and may reveal new approaches to reduce the morbidity of both infections.

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INCREASED LEVELS OF HIV TARGET CELLS AND VASCULARITY IN FEMALE GENITAL MUCOSA WITH *SCHISTOSOMA HAEMATOBIUM* INFECTION

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Schistosoma haematobium frequently causes lesions in the female genital mucosa. Studies suggest that female genital schistosomiasis may increase the risk of human immunodeficiency virus (HIV) transmission. However, the potential mechanisms for such an association have not yet been explored. The aims of this study are to quantify HIV target cells and blood vessels in female genital mucosa infected with *S. haematobium*. In a cross-sectional study, cervicovaginal biopsies of Malawian women (n=61) and controls were stained with antibodies to CD3, CD8, CD68 (macrophages), S100 protein (epithelial Langerhans cells), CD31 and vWF (endothelial cell markers). CD4+ T lymphocytes were identified from two consecutive CD3+ and CD8+ 3.5 µm thick sections. The density of CD4+ T lymphocytes was significantly higher surrounding calcified *S. haematobium* eggs, and the density of macrophages was significantly higher surrounding viable eggs compared to genital mucosal tissue without infection (p=0.034 and p=0.018, respectively). The density of epithelial Langerhans cells was not different between women with and without genital schistosomiasis (p=0.25). Tissue containing parasite eggs was significantly more vascularised (vWF) compared to healthy controls (p=0.017). Immunostain with CD31 identified significantly more granulation tissue surrounding viable compared to calcified eggs (p=0.032). In conclusion, the findings suggest that *S. haematobium* infection may cause changes that could increase HIV susceptibility in the female genital mucosa. Cervicovaginal mucosa with *S. haematobium* eggs contained more HIV target cells and was significantly more vascularised compared to genital mucosa without eggs. The association with calcified eggs may indicate that *S. haematobium* infection sustains a long-lasting increased HIV susceptibility in the female genital mucosa. Further studies are needed to explore the effect of anti-schistosomal treatment on lesions and cell populations in the genital mucosa.

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DIFFERENTIAL ANTI-MALARIAL IMMUNE RESPONSES IN *SCHISTOSOMA MANSONI* AND *PLASMODIUM COATNEYI* CO-INFECTED RHESUS MACAQUES

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Schistosomiasis and malaria are the two leading parasitic diseases worldwide. Areas endemic for schistosomiasis and malaria overlap in sub-Saharan Africa as well as other parts of the world. In a previous study, we observed that children harboring schistosomes are twice as likely to have detectable malaria parasitemia as children who have *Plasmodium falciparum* infection alone. To test whether a concurrent schistosome infection would exacerbate a malaria infection, we used the rhesus macaque model. Four macaques were percutaneously exposed to 500 cercariae of *Schistosoma mansoni*. At week eight post-infection, these macaques plus four additional animals were exposed to the bites of *Anopheles dirus* mosquitoes that were infected with *P. coatneyi.* Macaques with schistosomiasis developed higher parasitemia than macaques with malaria alone. In addition, anti-malarial drug treatment was more successful in macaques infected with malaria alone than macaques with schistosome co-infection, suggesting that schistosomiasis impairs the anti-malaria immune responses that are necessary for effective parasite treatment. To confirm this, we analyzed the malaria specific antibody responses in both groups of macaques and found that co-infected macaques displayed a significant impairment in their malaria specific antibody response. We also conducted studies to understand the role of the route of infection during co-infections. For these studies, we again exposed four macaques to 500 cercariae of *S. mansoni.* At eight weeks of infection, these macaques plus four additional animals were intravenously inoculated with 50,000 *P. coatneyi* blood stage parasites. These macaques were also given sub-curative doses of anti-malarial treatment throughout the infection. Analysis of parasitemia and antibody data demonstrate no significant differences between co-infected and malaria only infected macaques, suggesting that the route of malaria exposure might mediate the difference in immunological and pathological outcomes in schistosomiasis and malaria co-infections.

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SCHISTOSOME EGG ANTIGENS DIRECTLY ACTIVATE HUMAN TROPHOBLASTS

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Schistosomiasis infects ~ 40 million women of childbearing age, however, the impact of schistosome infection on the health of a pregnancy remains poorly understood. Previously, we demonstrated that schistosomiasis during pregnancy results in pro-inflammatory mediators in maternal, placental and fetal blood. Whether placental tissues themselves respond to schistosome infection remains unclear. In this study, we stimulated human trophoblast cells isolated from normal, healthy placentas (n=5) with endotoxin-free schistosome egg antigens (SEA) and assayed culture supernatants for inflammatory and fibrotic mediators. Trophoblasts were syncytialized prior to stimulation, as the syncytiotrophoblast is responsible for nutrient, gas, and waste exchange, is directly bathed in maternal blood, and therefore the most likely trophoblast cell type to respond to SEA. Of the analytes examined, secretion of interleukin (IL)-6 and IL-8 was significantly up-regulated in trophoblasts exposed to SEA compared to control treated cells (3.74 and 2.49 fold, respectively, both $P < 0.05$). Interestingly, IL-10, commonly associated with reactivity to SEA and critical in pregnancy, was unchanged. In addition, molecules involved in placental invasion and growth, including tissue inhibitor of metalloproteinase (TIMP)-2 and -3, both showed a trend toward increased expression following SEA exposure. Finally, key molecules involved in the availability of insulin-like growth factor (IGF), IGF binding protein-1 and -5 showed a trend toward increased expression with SEA exposure. These data suggest schistosome antigens directly activate human trophoblasts, resulting in increased production of pro-inflammatory cytokines, decreased availability of IGF, and altered remodeling of the placental environment. Enrollment is ongoing and we are extending our analyses to determine the trophoblast signaling pathways activated by SEA stimulation. This report is the first of its kind to examine the effect of SEA directly on the human placenta.

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A NOVEL ROLE FOR IGE IN HUMAN SCHISTOSOMIASIS

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Resistance to schistosomiasis is associated with increased levels of serum parasite-specific IgE. IgE exerts its functions through its cellular receptors, FcεRI and FcεRII/CD23; however its functional significance requires further characterization in humans. We previously reported that increased levels of CD23+ B cells correlate with resistance to schistosomiasis in hyper-exposed populations and sought to define their potential function and relationship with IgE. We found that CD23+ B cells are a heterogeneous B cell population with functional and phenotypic differences. Circulating CD23+ B cells are uniquely activated in schistosomiasis and express the CD23b isoform and CXCR5, the homing receptor for lymphoid follicles. High CXCR5 expression by CD23+ B cells was associated with the capacity to home to cognate ligand, CXCL13. CD23-bound IgE cross-linking increased surface expression of CXCR5 suggesting that CD23b+ B cells home directly into the lymphoid follicles upon antigen capture. Human schistosomiasis is an intravascular parasitic infection associated with a high antigenic burden in the blood. Thus, circulating CD23+ B cells likely capture and shuttle antigens from the blood directly to the splenic follicles through surface bound IgE, thereby highlighting a new function for both IgE and B cells. This process appears to play an important role in the development of protective immunity to schistosomiasis.

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THE HUMAN IGE RESPONSE TO TEGUMENTAL-ALLERGEN-LIKE PROTEINS IN *SCHISTOSOMA HAEMATOBIUM*

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The exact mechanisms mediating the development of human age-dependent immunity to schistosomiasis have yet to be determined. However, it is known that IgE levels against SmTAL1 (Sm22.6) correlate with resistance to *Schistosoma mansoni* reinfection in endemic areas. SmTAL1 is a member of the Tegumental-Allergen-Like protein family. Members of this family are characterized as having two EF hand domains (or pseudo EF hand domains) and a dynein light chain. The TAL proteins have a high level of sequence similarity, but very different developmental expression patterns. While the *S. mansoni* TALs are becoming relatively well characterized, very little is known about their *S. haematobium* homologues, and the *S. haematobium* genome has yet to be sequenced. Now, using a Sanger EST database, we have employed bioinformatic methods to predict the sequence of selected ShTALs and confirmed transcription with PCR using RNA from *S. haematobium* egg, cercariae and worm material. We have used RACE (Rapid Amplification of cDNA Ends) to determine their full-length sequences and qPCR to determine the developmental expression of these ShTALs in within the mammalian host. We have expressed 3 full-length ShTAL proteins as recombinants- ShTAL1 (predominantly worm), ShTAL2 (egg and worm) and ShTAL8 (egg). These recombinant proteins were used in isotype-specific ELISA assays with sera from 403 S. *haematobium*-infected patients from an endemic area of Mali in a cross-sectional treatment and reinfection study. IgG_1, IgG_4 and IgE antibody responses to these three ShTALs will be discussed in the context of the developmental expression of the ShTALs, as it is thought that the developmental expression pattern of the ShTALs will determine the qualitative and quantitative isotype responses of the infected population. The study of the ShTALs provides a unique insight into a much understudied parasite species, and the immune responses will help us understand the mechanisms underlying the age-dependent development of human resistance to schistosomiasis.

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CO-ADMINISTRATION OF PRAZIQUANTEL AND ALBENDAZOLE TO SCHOOL CHILDREN LIVING IN A UGANDAN COMMUNITY CO-ENDEMIC FOR *SCHISTOSOMA MANSONI* AND HOOKWORM: POST-TREATMENT PARASITE- AND ALLERGEN-SPECIFIC HUMORAL RESPONSES AND PATTERNS OF REINFECTION

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Chemotherapeuatic treatment of schistosomiasis in mono-endemic communities is often associated with post-treatment changes in parasite-specific antibody responses. Antibody responses to adult worm antigens generally increase after treatment, whilst those to egg antigens tend to decrease or are unchanged. In contrast to schistosomiasis, treatment of hookworm is generally associated with sharp declines in specific antibody responses. Since these two helminth infections frequently co-exist, WHO now advocates concurrent albendazole and praziquantel treatment. Recent studies have shown that the co-administration of these two drugs does not significantly alter their safety or efficacy, but little is known about the effects of combined treatment on post-treatment immune responses

and subsequent reinfection in co-endemic populations. Since there is also growing evidence that both parasites may protect against allergy, potential impacts on the incidence of allergic disease is another consideration. We investigated changes in total, parasite-specific and allergen-specific IgG_1, IgG_4 and IgE antibody responses following combined praziquantel and albendazole treatment of school-aged children living in a Ugandan community co-endemic for schistosomiasis mansoni and hookworm. Post-treatment changes in schistosome-specific and hookworm-specific antibody responses were consistent with observations from mono-endemic communities. Antibody responses to adult hookworm or *Schistosoma mansoni* egg antigens either decreased after treatment or were unchanged, whereas those to *S. mansoni* adult worm antigens increased. Post-treatment increases in IgE to adult worm antigens were associated with reduced susceptibility to *S. mansoni* reinfection, but there was little evidence for any antibody-mediated resistance to hookworm infection. There was some evidence for a negative association between schistosomiasis and allergy. Findings will help predict the public health effects of combined treatment, as well as providing insight into the biology of these two helminth infections and also allergic disease.

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APPLICATION OF NEW RECOMMENDATIONS FOR ASSESSING WHEN TO STOP MASS DISTRIBUTION OF AZITHROMYCIN FOR TRACHOMA

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To eliminate blinding trachoma, the World Health Organization recommends implementing the SAFE strategy which includes annual mass drug administration (MDA) with azithromycin. Current impact assessment guidelines suggest MDA may stop after 3 to 5 annual rounds where the prevalence of trachomatous inflammation follicular (TF) among children 1-9 years of age is below 5%, at the sub-implementation unit (the sub-district). We applied the current guidelines in 13/21 districts of South Wollo zone, Amhara Regional State, Ethiopia after 3 years of annual MDA to determine whether to stop MDA. Ten communities each were selected with a probability proportionate to population size in 36 sub-districts in the 13 districts. In each community, one development team (defined segment of the community) was randomly selected and all residents in all households were registered and those present were screened for clinical signs of trachoma. Overall, 38,852 residents were registered from 9,263 households of whom 33,800 (87.0%) were examined. District-level prevalence of TF in children aged 1-9 years ranged from 0.9% to 64.4% and sub-district prevalence ranged from 0.8% to 72.9%. A total of 6/36 sub-districts and 2/13 districts were below the threshold of 5% TF in children aged 1-9 years. Surveyed coverage of persons ever taking antibiotics ranged by district from 74.4% to 95.9% and coverage with 3 rounds of antibiotics ranged from 23.2% to 91.7%. The experience in South Wollo demonstrates that impact evaluation designed and powered to give a prevalence estimate at the sub-district level are possible. However, the scale of the work required excruciating attention to detail, was logistically challenging to implement, and produced a demanding data-entry load. Future impact assessments would be simplified by the use of electronic data collection. Most importantly, interpretation of the results is not as simple as stopping MDA in sub-districts below 5% given the proximity of hyper-endemic sub-districts. Further analysis of the spatial distribution of trachoma between sub-districts is needed.

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SUPPORT FOR INTEGRATED COVERAGE SURVEYS: EXPERIENCE FROM PLATEAU STATE, NIGERIA

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In Plateau State, volunteer community drug distributors (CDDs) who provided mass drug administration (MDA) for the established lymphatic filariasis elimination program were trained to register households for azithromycin MDA as part of the SAFE strategy to eliminate blinding trachoma. During the same period, LLINs were distributed from central points as part of an enhanced malaria control campaign. An integrated, cluster randomized survey was implemented in three districts to estimate the true coverage of MDA and LLIN interventions. Randomly selected households within a selected community were visited by a survey team and all household residents were enumerated. Residents who were present at the time of the interview were asked to report whether they did or did not take azithromycin for trachoma. Participation was verified using the drug distribution registers where available. Reported nets were observed and individuals were asked about net use. A total of 364 of 392 visited households were surveyed. From the surveyed households, responses were recorded for 1 858 out of 2 185 registered persons (85.0%). Overall, azithromycin was reported as taken by 56.5% (95%CI 42.9-70.2%) of surveyed individuals. Among households reporting to have received the drugs from a CDD, antibiotic coverage was 76.5% (68.0-84.9%). At least 2 nets were reported owned by 79.7% (69.8-89.7%) of households and 98.3% (96.9-99.7%) reported receiving the newest net from the campaign; 52.0% (39.5-64.5%) of households reported that the newest net was being used. The household coverage survey identified households that were not registered and therefore did not receive antibiotics. Coverage in future rounds of MDA can be enhanced by updating treatment registers to cover all households, and identifying and training new CDDs in unregistered (and untreated) communities. The national target of 2 nets per household was nearly achieved, but future efforts should focus on improving net use. This survey highlights the ease and importance of integrated monitoring of interventions through household surveys.

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MASS DRUG ADMINISTRATION FOR HELMINTHS WITH ALBENDAZOLE AND IVERMECTIN IN AN AREA ENDEMIC FOR *STRONGYLOIDES STERCORALIS*, ORAN, ARGENTINA

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Management of soil transmitted helminths (STH) in highly endemic communities is based on school-based mass drug administration (MDA) programs. Due to its unique characteristics, decisions on the optimal tools needed for evaluating prevalence, monitoring efficacy, selecting appropriate communities for intervention and determining the appropriate medications are further complicated when *Strongyloides stercoralis* (St

st) is included in the spectrum of targeted STHs. In 2010, a community-based MDA program was started in Orán, northwestern Argentina, an area highly endemic for STH (including St st) The goal of the program is to assess the performance of single dose combination therapy with albendazole and ivermectin, and to report the utility of a new recombinant antigen based ELISA for St st as a tool for assessing seroprevalence. The intervention population consisted of approximately 2400 individuals; 1200 from 3 rural communities, and an additional 1200 from 3 urban/peri-uban communities. Plantations in the rural area and street blocks in the urban/peri-urban area were used as the unit of randomization; 20% of the population was selected for sampling. The following parameters were assessed in each individual: single stool specimen analyzed through a comprehensive panel including sedimentation concentration, Harada-Mori, agar plate and Baermann techniques; St st NIE-ELISA serology and hemoglobin. We present the preliminary results of the initial pilot intervention in one rural and one urban community. The overall prevalence of STH by stool examination was found to be 32%, with 12% positive for St st. Sensitivity increased to 31% for St st when NIE-ELISA was included. A total of 864 individuals from 2 communities with a population of 1127 persons were treated with ivermectin and albendazole. Active and passive surveillance revealed no significant (Grade 3 or 4) adverse events. Only 3 individuals refused treatment. This initial MDA comprehensively targeting STH with the added use of St st serology for prevalence calculations is a promising approach with an appropriate safety and efficacy proven regimen. Further inclusion of the remaining communities and rounds of treatment will provide valuable information for defining a strategy for the management of STH in highly endemic communities.

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TWO YEARS EXPERIENCE OF INTEGRATED MASS DRUGS ADMINISTRATION OF NTD CONTROL IN TANZANIA MAINLAND

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Tanzania has embarked in an integrated control of Neglected Tropical Disease (NTD) since 2009. Preventive Chemotherapy (PCT) targeted Diseases endemic in Tanzania are Lymphatic filariasis (LF), Onchocerciasis, Trachoma, schistosomiasis as well as STH. These diseases overlap throughout the country. Programme activities include; Social mobilization, training, drug delivery, data collection and compilation all in an integrated manner. Implementation Unit (IU) for control activities is districts councils and since there are over 140, a phased scale up is adopted to reach full geographical coverage by 2013. Coordination of the NTD control is by the government and implementation is in a cascade manner. In the past 2 years the programme has achieved increased therapeutic as well as geographic coverage towards integrated NTDs control. In 2009, working in 36 IUs (27% geographical coverage) a total of 2500 Health Workers and 28,000 drug distributors were trained. Advocacy and community mobilization reached 9700 influential people and decision makers. About 10 million at risk population were treated. With increased partner involvement and funding in 2011 two phased scale up approach will increase the geographical coverage to 75 (56% geographical coverage) and reaching out to 21 million at risk population by the end of year 2011. An integrated training manual, IEC materials and monitoring and evaluation tools have been developed and used in the field. The profile of NTDs is scaled up as it is now reflected into the Health Sector Strategic Plan III, a reference document of the Ministry. With the support of WHO, a master plan for NTDs is prepared and pharmacovigilance manual has been prepared. Despite the achievements, several challenges were encountered; these include; poor coordination among stakeholders, disease specific focus, low urban coverage, lower attention to non PCT NTDs as well as non PCT activities like surgeries. Benefits of Integration outweigh the challenges and it's a country's goal to achieve full and effective integration soonest possible.

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ACCURACY OF COVERAGE SURVEY RECALL FOLLOWING AN INTEGRATED MDA FOR LYMPHATIC FILARIASIS, SCHISTOSOMIASIS AND SOIL-TRANSMITTED HELMINTHIASIS

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Household-based coverage surveys are an important means of ensuring that mass drug administrations (MDA) for several neglected tropical diseases reach target coverage levels. Such surveys are used for validating coverage reported by drug distributors because the two methods are independent of each other; coverage surveys do not depend on accurate reporting or denominator estimates. A potential disadvantage of coverage surveys, however, is recall bias. We tested recall accuracy in surveys conducted 1, 6, and 12 months after an MDA in Togo, in which three drugs (albendazole, ivermectin, and praziquantel) were distributed. Independent observers ensured all MDA treatments were accurately recorded in registers for comparison with survey responses. A unique sample of compounds (household groups) was systematically selected for each survey. All compound residents (mothers answered for children <10 years of age) were shown examples of pills given during the MDA and asked which they had swallowed. Responses from 506, 1139, and 963 persons at the 1, 6, and 12 month surveys, respectively, were analyzed. Coverage among these persons (defined as having taken at least one MDA drug) was 88%, 87%, and 79%, respectively, according to MDA registers (the lower result at 12 months is likely an artifact due to poor matching of respondents to the MDA register data). Coverage estimates based on respondent recall were 88% (95% confidence interval [CI] 86-91%), 91% (CI 89-93%), and 90% (CI 87-91%), respectively. Concordance between respondent recall and register data was >93% at 1 and 6 months. Respondents generally distinguished between pills similar in appearance; 97% of those taking albendazole (large, white, rectangular, maximum dose 1 tablet) reported taking ½ or 1 tablet, while only 32% reported taking only 1 praziquantel tablet (large, white, oval, dose range 1-5 tablets). Concordance for correct recall of individual medications was >80% in all surveys, while concordance for correctly remembering pill dosage ranged from 41% to 85%. In this population, CS provided accurate and consistent estimates of overall coverage for up to one year following an integrated MDA. These data confirm the usefulness of CS in monitoring and evaluation of MDA, and suggest that CS might be postponed for up to one year without compromising recall accuracy, which might allow for integration into periodic large, multipurpose surveys.

1575

A SPATIAL APPROACH TO QUANTIFYING THE WATER SUPPLY, SANITATION AND HYGIENE (WASH) - HELMINTH INFECTION-ANAEMIA CAUSAL PATHWAY IN CHILDREN IN SUB-SAHARAN AFRICA

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Inadequate water supply, sanitation and hygiene (WASH) are well known risk factors for infections with schistosomes and soil-transmitted helminths. Urinary schistosomiasis and hookworm infections are known to cause anaemia which is a severe public health problem in most countries of sub-Saharan Africa. Using a novel, spatial analytic approach, we aimed to quantify for the first time the role of WASH in the risk of *Schistosoma haematobium*, *S. mansoni* and hookworm infection in school age children in West Africa; estimate the risk of anaemia in children

aged 1-4 y (preschool children) attributable to malnutrition, malaria, and helminth infections; and estimate the number of anaemia cases in preschool children for 2011. We generated predictive maps showing probability of absence of WASH. These predictions were then used as covariates in Bayesian geostatistical models for the three helminth species. Bayesian geostatistical models were subsequently developed to predict the geographical distribution of anaemia of preschool children, adjusting for their nutritional status, predicted *Plasmodium falciparum* parasite rate in the 2- to 10-y age group (*Pf* PR_{2-10}), and predicted prevalence of *S. haematobium* and hookworm infections. We estimated attributable fractions of water supply for *S. mansoni* and *S. haematobium* to be 47% and 71% respectively. The attributable fraction of natural floor type was 21% for *S. haematobium*, 16% for *S. mansoni* and 86% for hookworm. An estimated 36.8%, 14.9%, 3.7%, 4.2%, and 0.9% of anaemia cases could be averted by treating malnutrition, malaria, *S. haematobium* infections, hookworm infections, and *S. haematobium*/hookworm coinfections, respectively. We estimate that in 2011, approximately 6.7 million children aged 1-4 y are anaemic in the three study countries. This work identified communities in West Africa where preventive chemotherapy integrated with interventions to improve WASH will yield the greatest health benefits. It also identifies the geographical limits of anaemia burden and the contribution that malnutrition and parasites make to anaemia.

1576

ESTIMATING COSTS AND FUNDING GAPS OF INTEGRATED NTD CONTROL PROGRAMS: THE FUNDING GAP ANALYSIS TOOL (FGAT)

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With neglected tropical disease (NTD) control programs being increasingly integrated and expanded, it is critical to improve the accuracy of estimated activity costs and funding gaps for budget and planning purposes. Moreover, there is a growing need to better understand the cost drivers of implementing integrated control in order to determine an efficient allocation of available resources. To meet these objectives, the Funding Gap Analysis Tool (FGAT) was developed by the USAID funded NTD Control Program, led by RTI International with WHO endorsement, to assist national NTD programs to organize and analyze activity costs concordant to their national plans of action. To date, the FGAT has been completed by Ministries of Health in over a dozen countries, revealing several valuable outcomes. The FGAT allows countries to recognize cost efficiencies that can be achieved by integrating high cost-driving activities such as training, drug delivery, and social mobilization between disease programs. It also permits countries to calculate the effect of a significant increase or decrease in key costs, such as fuel or medicines, on the program as a whole. Furthermore, the FGAT can direct funding into specific program areas by identifying gaps at the sub-activity and district levels that may be undetected at higher levels alone. Such results ultimately lead to more precise budgets and standardized year-to-year cost estimates. Additionally, the FGAT provides NTD programs data-related ancillary benefits including a referential database for analysis and summarized outputs for donor advocacy. In the longer term, the data collected by FGAT will allow for in-depth longitudinal and cross-country cost analyses in tandem with program evaluation data. These findings will be highly beneficial for guiding cost-effective decisions and practical plans of action for integrated NTD control programs.

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DISSECTING HUMAN ANTIBODY RESPONSE AGAINST DENGUE VIRUS USING REVERSE GENETICS

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Human exposed to dengue virus (DENV) develop a type specific neutralizing antibody (nAb) response against the infecting serotype. However, the epitopes recognized by these nAbs in polyclonal sera are not known. Here, we describe a reverse genetic approach to map the sites on the DENV envelope (E) protein that are targeted by nAbs in human sera. Mouse monoclonal antibodies (mAbs) that strongly neutralize DENV type specifically bind to a unique epitope located on the lateral ridge of the domain III (EDIII) of the E protein. Sub complex mouse mAbs that neutralize more than one DENV serotype, but not all the four serotypes, target an epitope located on the A strand of the EDIII. We have used bacterially expressed recombinant EDIII (rEDIII) protein to deplete the EDIII reactive antibodies from polyclonal sera and demonstrated that EDIII antibodies make only a minor contribution to the neutralization potency of human dengue immune sera. One potential problem with this approach is that it may not deplete all EDIII reactive antibodies because of folding differences between the recombinant protein and the native protein on the virus. We used recombinant DENV2 viruses that contain mutations on the lateral ridge (FG loop) and A strand epitopes (AA positions at 305,307,310), to measure the contribution of human EDIII reactive antibodies in type specific neutralization. The mutant viruses escaped from neutralization by mouse mAbs that bind to lateral ridge and A strand epitopes. However, the 50% neutralization titers of human immune sera were similar for wild type and EDIII mutant viruses. In agreement with previous EDIII depletion studies, our results indicated that EDIII epitopes targeted by mouse neutralizing antibodies were not the target of the human neutralizing response. We hypothesize that nAbs in human sera mainly target sites located on the domain I /II of the E protein. We have identified putative binding sites on the EDI/II and currently experiments are in progress to mutate these sites on DENV3 and DENV2 E protein to test the hypothesis.

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DENV-3 GENOTYPE SPECIFIC NEUTRALIZATION BY HUMAN POLYCLONAL SERUM AND HUMAN MONOCLONAL ANTIBODIES

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Dengue viruses (DENV) are enveloped single-stranded positive-sense RNA viruses transmitted by *Aedes aegypti* and *A. albopictus* mosquitoes. There are four genetically distinct serotypes designated DENV-1 through DENV-4. DENV-3 is further subdivided into four distinct genotypes designated I-IV. The dengue scientific community has long contended that infection with one serotype confers lifelong protection against subsequent infection with the same serotype, irrespective of virus genotype. However this hypothesis has never been rigorously tested and the role of DENV genotypic variation in protection from repeated infection is unknown. Recent studies have shown that *in vitro* neutralization titers vary substantial by DENV genotype tested and low titers (<1:50) may not be protective. To better understand the role genotypic variation plays in DENV-3 neutralization and protection, we previously designed and constructed a panel of isogenic, recombinant DENV-3 infectious clones, each expressing a representative envelope glycoprotein (E) from a different DENV-3 genotype. When the recombinant

viruses were tested in neutralization assays using human homotypic primary dengue immune sera, neutralization titers ranged varied by as much as 10-fold, depending on the genotype of the E protein expressed by the virus. The observed variability in neutralization titers suggests that relatively few residue changes in the E glycoprotein may have significant effects on DENV specific humoral immunity and may influence antibody mediated protection in the setting of both natural infection and vaccination. To further explore the role of genotypic variation, six anti-DENV-3 human mAbs were tested against the clone panel. One mAb exhibited strong genotype-specific neutralization variability. Using recombinant E protein, site-directed mutagenesis in the parent clone background and the generation of mAb escape mutants against this mAb the genotype specific amino acid residues were mapped on the E glycoprotein. The DENV3 recombinant virus panel described here is a useful tool for mapping antibody responses and assessing the breadth of natural and vaccine induced immune responses.

1579

ANALYSIS AND IDENTIFICATION OF EPITOPES ON DENGUE VIRUS ENVELOPE PROTEIN RECOGNIZED BY MONOCLONAL ANTIBODIES AND POLYCLONAL HUMAN SERA BY A HIGH THROUGHPUT ASSAY

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The envelope (E) protein of dengue virus (DENV) is major target of neutralizing antibodies and dengue vaccine development. While previous studies on domain III or domain I/II alone have reported several epitopes of monoclonal antibodies (mAbs) againt DENV E protein, several questions including the possibility of interdomain epitopes, the relationship between epitopes and binding specificity as well as neutralizing potency remains largely unexplored. We developed a high throughout dot blot assay by using a panel of 67 alanine mutants of predicted surface-exposed E residues as a systemic approach to identify epitopes recognized by 12 mouse mAbs and polyclonal human sera, followed by confirmation with capture-ELISA. Three mAbs were found to recognize a novel epitope involving residues at domain II central interface, and three mAbs recognized residues at both domain III and lateral ridge of domain II, suggesting the presence of interdomain epitopes. Analysis of the conservation index of each epitope residue and conservation score of 91 mouse mAbs reported thus far revealed that the conservation scores correlated with the binding specificity. Compared with mAbs generated by traditional protocol, the potent neutralizing mAbs generated by a new protocol recognized multiple residues in A strand or residues in C strand/CC' loop of DENV2 and DENV1, as well as multiple residues in BC loop and residues in DE loop, EF loop/F strand or G strand of DENV1. These findings have implications for future development of epitope-specific diagnostics and epitope-based dengue vaccine. Moreover, the predominant epitopes of anti-E antibodies in polyclonal sera were found to include fusion loop residues and some non-fusion residues in the same or adjacent monomer, adding to our understanding of humeral immune responses to DENV at the epitope level.

1580

MODULATION OF HUMAN INNATE IMMUNITY BY SRI LANKAN DENGUE-3 VIRUSES ASSOCIATED WITH DENGUE FEVER AND DENGUE HEMORRHAGIC FEVER

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Dengue Virus (DENV) is the leading mosquito borne viral threat in the world with 50 to100 million people infected and 2.5 billion people at risk annually. Infection by one of the four serotypes of DENV can clinically present as dengue fever (DF), a febrile illness or the more severe dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS), which is mostly observed in people experiencing a secondary DENV infection. (DF is very common following primary infections.) There are several potential reasons as to how these more severe secondary infections arise including antibody dependent enhancement (ADE), T-cell mediated immunity, and differences in viral genotypes. The emergence of a new strain of DENV3 in Sri Lanka in 1989 has been associated with an increase in disease severity, as reported previously. Our project aims to determine if there are intrinsic differences in the ability of Sri Lankan DENV3 strains isolated before and after the emergence of DHF to infect human immune cells. We obtained DENV-3 viruses derived from primary isolates from Sri Lanka and analyzed their replication and innate immune phenotype in a primary human system. Using monocytes and monocyte derived dendritic cells (DCs) we studied virus replication, infectious particle release and as well as the innate immune responses in infected cells by plaque assay, qRT-PCR and multiplex ELISA. Our data shows an increase in interferon (IFN) and IFN -stimulated gene induction in DCs from multiple donors infected with post-DHF associated DENV-3, compared to the pre-DHF associated DENV-3. Infections of DCs with our DENV-3 isolates in the presence of DENV + sera are ongoing to analyze their phenotype in an ADE context. Future work will also investigate specific mutations in the DENV NS2B3 and NS5 genes may correspond to some of the observed differences in virulence between the viruses.

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ROLE OF ACUTE B CELL RESPONSE IN DENGUE DISEASE SEVERITY AND LONG-TERM IMMUNITY

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Dengue, caused by four dengue virus serotypes (DENV-1-4), is the most prevalent mosquito-borne viral disease in humans, causing classic dengue fever (DF) and dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS). While severe disease has been associated with heterotypic secondary DENV infection mediated by antibody-dependent enhancement and stimulation of cross-reactive T cells, the vast majority of secondary infections result in mild or asymptomatic disease, suggesting a protective role of the cross-reactive immune response. Little is known about the acute B cell response during DENV infection and its impact on the breadth and quality of the long-term humoral response. During the 2010 epidemic, we studied the B cell response in 193 children suspected of DENV infection who were enrolled in a hospital-based study in Managua, Nicaragua, including 127 DENV-positive cases and 66 Other Febrile Illnesses (OFI). The dominant serotype was DENV-3 (82.6%). Sixty-four patients had a primary DENV infection, 54 experienced a secondary infection and 5 cases were undetermined. We measured by flow cytometry the percent of naïve and memory B cells and plasmablasts (PB)/plasma cells (PC) in fresh whole blood and demonstrated a significant increase in PB/PCs in DENV-positive cases when compared to OFI (1.5+/-0.2; n=83 *vs.* 0.6+/-0.2;

n=40; p=0.003). No significant difference was found among naïve and memory B cell compartments. These data correlated with the number of DENV-specific antibody (IgG)-secreting cells measured by ELISpot *ex vivo* (representing the circulating PC) and after polyclonal *in vitro* stimulation (representing the circulating memory B cells). After a secondary DENV infection, we found a mean of 2,046 DENV-specific PC/10^6 PBMCs (14.7% of total IgG) and 870 DENV-specific memory B cells/10^6 PBMCs (3.8% of total IgG). We are currently measuring the DENV-specific neutralization capacity of the sera, using a flow cytometry-based assay, as well as the avidity of the sera in longitudinal samples (3, 6 and 12 months after infection) using a competition-based ELISA with recent Nicaraguan DENV strains. The breadth of the acute B cell response will be correlated with severity of disease and the neutralization capacity and avidity of the serum. This study improves our knowledge of B cell and long-term humoral responses to DENV infection, which could ultimately impact the design of safe and effective dengue vaccines.

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RISK FACTORS OF DENGUE HEMORRHAGIC FEVER IN A PREDOMINANT DENGUE SEROTYPE 2-INFECTED CASE-CONTROL STUDY

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Dengue, an arthropod-borne virus infection, is of serious concern in tropical and subtropical regions. Dengue hemorrhagic fever (DHF) is a severe form of dengue where several risk factors e.g white race, female gender, dengue serotype 2, allergy, diabetes and hypertension were reported in different studies. In our 2004 retrospective study where serotype 1 predominated, gender, ethnicity and comobidities were not significantly associated with DHF. Our current retrospective case-control study explores these risk factors in 2006 and 2007-2008 adult dengue cases in Singapore where serotypes 1 and 2 predominated respectively. In this study, 149 DHF and 326 DF from the year of 2006 and 669 DHF and 1141 DF from the year of 2007-2008 were included. We performed univariate descriptive analysis, and adjusted measures for the association were estimated using multivariate logistic regression adjusting for potential confounding variables. Results from 2006 data, similarly in 2004 study, showed no significant association for gender, comobidities and ethnicity with DHF. In contrast, results of 2007-2008 showed age groups 30-39 years (adjusted OR= 1.41, p=0.008) and 40-49 years (adjusted OR=1.34, p=0.042), female gender (adjusted OR=1.56, p<0.0001), Chinese race (adjusted OR=3.13, p<0.0001), diabetes (adjusted OR=1.82, p=0.016) and diabetes with hypertension (adjusted OR=2.16, p=0.013) as risk factors for DHF. This study also suggested that reported risk factors for DHF may differ between serotype 1 and 2. In conclusion, older Chinese female patients with both diabetes and hypertension may be at greatest risk for DHF when serotype 2 predominates.

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MODELLING DENGUE PATHOGENESIS, CONSIDERING DIFFERENCES BETWEEN PRIMARY AND SECONDARY INFECTION

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We describe a mathematical model of dengue virus infection within an individual, parameterised with sequential viraemia data. In fitting this model to multiple patients' data, we are able to consider what explains differences seen between people, between primary and secondary infection and possibly between different disease severities. We assess the factors explaining the timing of the peak of virus and the rate of virus decline seen. In addition, though we are missing data in the early stages of infection, as we are using hospitalised cases, we also look at the determinants of the rate of increase of viraemia. We find that differences in the modelled immune response growth can recreate the different virus curves of individuals. We see differences in estimated model parameters between primary and secondary infection. We identify possible differences in the efficiency of virus entry to the cell, initial number of antigen recognising immune cells and the efficacy of the immune response as potential explanations of differences between primary and secondary infections. Incorporating immunological data into our inferential framework, we are able to look more in depth at the immunological processes governing infection and the magnitude of these processes in different people. This suggests differences in pathogenesis which are correlated with primary and secondary disease, and provides more insight into the interplay between virus and immune dynamics. This initial attempt aims to develop a mathematical model of pathogenesis which is tightly coupled to data to describe dengue infection dynamics. As well as helping in the understanding of the infection process, greater understanding of these processes and their timing could help to understand the transmission of the virus and the impact of control measures. We illustrate this by considering the potential impact of antiviral drugs on dengue infection and transmission.

1584

DIFFERENCES IN INHERENT SUSCEPTIBILITY TO *PLASMODIUM FALCIPARUM* AND RATES OF NATURAL INFECTION IN POPULATION SUBGROUPS OF *ANOPHELES GAMBIAE*

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Understanding and characterizing how population subgroups within *Anopheles gambiae* s.s. differ in their susceptibility to *P. falciparum* infection is important for the development of better and more exacting control measures. We combined experimental laboratory infections and capture of naturally infected adult mosquitoes in order to address two important questions (1) differences in the inherent genetic susceptibility to malaria parasites, as measured by experimental infection with gametocytes, and (2) differences in natural infection rates amongst wild caught female mosquitoes. The latter approach summarizes natural variables such as behavior, mosquito age, and bloodmeal source, whereas the former approach controls for these variables. Membership in population subgroups was determined by genotyping.

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TRANSPOSON-BASED "FORWARD" GENETICS IN *ANOPHELES STEPHENSI*

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Transposons can be useful for not only shuttling transgenes into genomes but, under the right conditions, also for identifying and isolating genes. Transposon-based gene- and enhancer-trap technologies are particularly powerful because they permit the identification of interesting genes or enhancers to be recognized based on spatial and temporal patterns of reporter gene expression from the transposon. In the case of transposon-

based gene-traps the transposon insertion site is in the gene responsible for the observed pattern of reporter gene expression, allowing the gene to be easily isolated. Here we report data showing the feasibility of performing transposon-based enhancer- and gene-traps in *Anopheles stephensi* using the *piggyBac* transposon. Transgenic lines with *piggyBac* elements containing an enhanced cyan fluorescent protein (ECFP) gene under the regulatory control of the 3xP3 promoter were remobilized after crossing to a transgenic line expressing *piggyBac* transposase. Germ-line transpositions were detected in approximately 1% of the progeny. The activity of the 3xP3 promoter is sensitive to changes in its position within the genome and can detect the presence of local enhancers and promoters. A diverse collection of transgenic lines was made in which ECFP expression was in interesting and useful temporal and spatial patterns demonstrating the potential of this approach. This technology promises to create new opportunities to investigate insect/pathogen interactions.

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GENETIC ANALYSIS OF PREFERENCE FOR HUMAN SCENT IN THE DENGUE FEVER MOSQUITO, *AEDES AEGYPTI*

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An ancestral, forest form of the Dengue Fever Mosquito, *Aedes aegypti*, prefers the odor of non-human animals, while a more recently evolved, domestic form strongly prefers human odor. Classic work from the 1970's and 1980's showed that these two forms coexist in several places along the coast of East Africa, where they maintain their ecological differences despite being fully interfertile. This situation provides an unusual opportunity to examine the genetic basis of traits that adapt mosquitoes to humans. We have verified the continued coexistence of human and animal-adapted mosquito populations in the Rabai region of Kenya, established laboratory colonies, and documented striking, genetically-based differences in preference for human *vs*. animal scent. We are now using high-throughput transcriptome sequencing to identify candidate genes in the three mosquito tissues involved in the reception and processing of odor cues: the antenna, maxillary palp, and brain.

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AEDES AEGYPTI FORMOSUS IS NOT A SINGLE SUBSPECIES IN SENEGAL, WEST AFRICA

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Aedes aegypti is the major vector of the Dengue, Yellow Fever, and Chikungunya viruses. The subspecies *Aedes aegypti aegypti (Aaa)* has a global distribution while *Aedes aegypti formosus* (*Aaf*) is limited to Sub-Saharan Africa. We used McClelland's scoring of the first abdominal tergite to subdivide *Aaf* collected in Senegal into form *"F"* (no white scales) and form *"G+" (*white scales present). Average fecundity was observed within *F* or *G+* forms but few eggs and very few larvae were produced in *F* x *G+* crosses. Analysis of the *Argo2* gene identified distinct SNPS associated with *F* or *G+* forms. "F" markers predominated in the southeastern, forested regions of Senegal and the "G+".markers predominated in western and northern Senegal. Analysis of genotype frequencies demonstrated that 29.2% (31/106) of tests departed significantly from HWE and 25 of the 31 significant tests had an excess of homozygotes. Wahlund's effect is one explanation for this and occurs when a collection is sampled as a single reproductive pool, when there are actually two or more reproductively isolated populations. When the Aaf "G+" were analyzed separately, genotypes fit HWE. However, when Aaf "F" was analyzed, an excess of homozygotes continued to be observed; suggesting that the "F" form may consist of more than one reproductively isolated subspecies. Linkage disequilibrium analysis of SNPs in cDNAs was performed on 10 different populations from Senegal, and 10 populations from throughout the world. There was a large amount of disequilibrium among alleles on all three chromosomes. We tested for suppression of recombination associated with inversions that had been previously detected in Aaf. We crossed *"F"* males with Higgs White Eye (HWE) Aaa females and then backcrossed male F_1 to HWE females. We anticipated finding suppressed recombination or that the *we* locus would be 14cM from the Sex locus as previously reported. Instead the loci were 14cM apart in some families but unlinked in others suggesting a translocation. All of these results suggest that *Aaf* is comprised of multiple subspecies in West Africa that can be distinguished based upon chromosomal polymorphisms and molecular genetic markers.

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POTENTIAL GENOTYPIC IDENTIFICATION OF THE BELOW GROUND PHENOTYPE IN THE *CULEX PIPIENS* COMPLEX IN CALIFORNIA USING SNP GENOTYPING

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The California West Nile virus vector is the *Culex pipiens* s.l. complex, which displays an array of behavioral adaptations and morphological differences that make efforts to reduce their numbers challenging. It is unclear if the observed variation is associated with genetically distinct populations, differing taxa, or if it represents a high degree of polymorphism within a single panmictic unit of randomly mating individuals. The current consensus in California is that the two nominal members, "quinquefasciatus" (south of 39°N) and "pipiens" (north 36°N) and their respective hybrids (between 39° and 36°N), occur in California. Because they vector several zoonotic diseases (e.g., dog heartworm, avian malaria, avian pox virus), the *Cx. pipiens* complex is receiving increasingly more attention related to defining genetic determinants and variations in their distinct phenotypes (e.g, blood feeding preference, oviposition behavior). We hypothesized that a more accurate structure within California can be determined using phenotypes as distinct populations opposed to geographically distinct populations; consequently, we used single nucleotide polymorphisms (SNPs) to identify the population structure of *Cx. pipiens* from five counties using above and below ground collection sites as the structuring phenotype. Our preliminary SNP analysis corroborates other studies; that there is evidence of a single panmictic population with sub-structuring within the state. This may explain the observed variations in distribution and behaviors of this complex. On average, we found a SNP every 7.5bp. Of interest, one genotype was found only below ground suggesting this genotype may be associated with oviposition site, breeding site and/or the *Cx. molestus* sub-species. Phylogenetic analyses identified this below ground genotype as a population distinct from *Cx. quinquefasciatus*, but genetically similar to *Cx. pipiens*. Additional SNP population genetic analyses will further resolve the systematic issues and define the population structure. Analyses based on larger sample sizes and collections from multiple trapping methods and separation based on additional phenotypes will confirm our data to determine the true nature of *Cx. pipiens* in California. These results provide more information on the roles that each potential member or population serves as zoonotic and epizootic vectors.

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IMAGINAL DISCS - A NEW SOURCE OF CHROMOSOMES FOR GENOME MAPPING OF THE YELLOW FEVER MOSQUITO *AEDES AEGYPTI*

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The mosquito, *Aedes aegypti,* is the primary global vector for dengue and yellow fever transmission. Sequencing of the *Ae. aegypti* genome has stimulated research in vector biology and insect genomics. However, the current genome assembly is highly fragmented with only ~30% of the genome being assigned to chromosomes. A lack of reliable source of chromosomes for physical mapping has been a major impediment to improving the genome assembly of *Ae. aegypti*. We propose to use mitotic chromosomes from imaginal discs of a 4th instar larva for cytogenetic studies of *Ae. aegypti*. High numbers of mitotic divisions on each slide preparation, large sizes and reproducible banding patterns of the individual chromosomes simplifies cytogenetic procedures. Based on the banding structure of the chromosomes, we have developed ideograms for each of the three *Ae. aegypti* chromosomes and placed 10 BAC clones and a 18S rDNA probe to precise chromosomal positions. The study identified imaginal discs of a 4th instar larva as a superior source of mitotic chromosomes for *Ae. aegypti*. The proposed approach allows precise mapping of DNA probes to the chromosomal positions and can be utilized for obtaining high-quality genome assembly of the yellow fever mosquito.

1590

WOLBACHIA-DENGUE INTERACTIONS IN *AEDES* MOSQUITOES

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Wolbachia are maternally transmitted Gram-negative intracellular bacteria and infect up to 65% of insect species. The ability of these alphaproteobacteria endosymbionts to induce the reproductive abnormality called Cytoplasmic Incompatibility (CI) has led to a large effort on developing *Wolbachia* as a novel genetic strategy for control of vector-borne diseases. We recently observed that *Wolbachia* induce resistances to dengue virus in *Aedes aegypti*. This provides *Wolbachia* a "mosquito vaccine" like feature, which can be introduced, driven through CI, and spread over mosquito population to block transmission of mosquito-borne diseases. We will present our recent works on how *Wolbachia*-mosquito interactions activate Toll pathway and induce expression of anti-dengue effectors. The mosquito gene expression profile regulated by *Wolbachia* will be dissected in the presence and absence of dengue virus, with special focus on immune and redox genes. We will also present evidences to show why *Wolbachia*-mediated viral interference does not present in *Ae. albopictus*, which naturally carries a *Wolbachia* superinfection. A *Wolbachia*-density dependent viral inhibition will be further illustrated using both mosquitoes and cell lines. We will discuss how our results will provide novel opportunities to develop environmentally friendly biopesticides or novel strategies for future control of vector-born diseases.

1591

INTRA-VITAL IMAGING REVEALS MULTIPLE MODES OF *PLASMODIUM* LIVER-STAGE ELIMINATION BY CD8+ T CELLS

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CD8+ cytotoxic T lymphocytes (CTL) can mediate protection against malaria by eliminating *Plasmodium* parasites in hepatocytes. To gain new insights into this anti-parasitic activity, we used spinning-disc confocal microscopy to visualize the CTL-mediated killing of GFP-labeled parasites *in vivo*. Killing was indicated by a profound decrease in the fluorescence of most of the parasites associated with at least one CTL. Strikingly however, most dying parasites were surrounded by clusters of multiple CTL. A variety of different modes of parasite death could be distinguished, ranging from the rapid loss of GFP signal to a progressive attrition of parasite fluorescence. CTL-parasite interactions generally lasted for the entire duration of imaging (mean of ~1 death event / 3 h of recording). These data are consistent with redundant mechanisms being used for the killing of parasites. In conclusion, the action of CTL on *Plasmodium* liver stages is a complex, multi-cellular killing process rather than a rapid and discrete event.

1592

S1P IS ASSOCIATED WITH PROTECTION IN HUMAN AND EXPERIMENTAL CEREBRAL MALARIA

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Cerebral malaria (CM) is associated with excessive inflammatory responses and endothelial activation. Sphingosine 1-phosphate (S1P) is a signaling sphingolipid implicated in regulating vascular integrity, inflammation and T cell migration. We hypothesized that altered S1P signaling during malaria contributes to endothelial activation and inflammation, and show that plasma S1P levels were decreased in Ugandan children with CM compared to children with uncomplicated malaria. Using the *Plasmodium berghei* ANKA model of experimental CM (ECM), we demonstrate that humanized S1PL-/- mice with reduced S1P lyase activity (resulting in increased bio-available S1P) had improved survival compared to wild type littermates. Prophylactic and therapeutic treatment of infected mice with compounds that modulate the S1P pathway and are in human trials for other conditions (FTY720 or LX2931) significantly improved survival in ECM. FTY720 treatment improved vascular integrity as indicated by reduced levels of sICAM, increased Ang1 (regulator of endothelial quiescence) levels, and decreased Evans blue dye leakage into brain parenchyma. Furthermore, treatment with FTY720 decreased IFNγ levels in plasma as well as CD4+ and CD8+ T cell infiltration into the brain. Finally, when administered during infection in combination with artesunate, FTY720 treatment resulted in increased survival to ECM. These findings implicate dysregulation of the S1P pathway in the pathogenesis of human and murine CM and suggest a novel therapeutic strategy to improve clinical outcome in severe malaria.

1593

ANTIBODY ENHANCED INTRACELLULAR KILLING OF *LEISHMANIA AMAZONENSIS*

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Experimental footpad infection of C3HeB/FeJ mice with the causative agent of disseminated cutaneous leishmaniasis, *Leishmania amazonensis,* leads to a chronic disease with high parasite load and large footpad lesion size that persists even after induction of an antigen-specific CD4+ Th1 host immune response. However, these lesions will heal if the animal is co-infected with *L. major*. We have used this fact to explore the immune factors that are capable of limiting *L. amazonensis* during coinfection and found that B cells and specifically their antibodies play an important role in killing this intracellular protozoan parasite. Using an *in vitro* assay with draining lymph node cells from infected animals we found IFN-γ receptor, iNOS, NADPH oxidase and FcR γ -common chain were required to kill *L. amazonensis* within infected macrophages. PI3 kinase-dependent superoxide production was detected late during the assay. We hypothesized that small soluble immune complexes could provide a late source of effector molecules for the NADPH oxidase pathway. Using soluble cross-linked non-specific IgG2a antibodies we were able to recapitulate parasite killing without immune cells from infected animals. Analysis via Image Stream flow cytometry and confocal microscopy indicated that non-specific antibody did not opsonize the parasite. We conclude that small soluble IgG2a immune complexes indirectly promoted parasite killing via superoxide production within infected macrophages. The vast majority of *Leishmania* infection studies show that during an ineffective immune response antibodies limit macrophage activation and promoted high parasite loads. Our studies add to this knowledge but indicate that during Th1 immunity antibodies can promote an enhanced macrophage microbicidal response and play a significant role in supporting T cell-mediated immunity.

1594

REGULATORY CD4+CD25^{HIGH} T CELLS FROM INDETERMINATE PATIENTS WITH CHAGAS DISEASE CAN SUPPRESS THE EFFECTOR CELLS AND CYTOKINES AND REVEAL AN ALTERED CORRELATION WITH THE DISEASE SEVERITY

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Several studies in Chagas disease demonstrate that immunoregulatory mechanisms control the intense immune activity in the chronic phase, preventing a deleterious effect of the excessive immune stimulation. Recently, the identification of the human CD4+CD25^{high} FOXP3+T cells (Treg) and its role have been the object of intense studies due to the putative critical role of these cells in maintaining self tolerance, as well as in the control of immune response. In this study we evaluated the phenotypic profile and the mechanisms by which Treg cells works in patients with the indeterminate (IND) and cardiac (CARD) clinical forms of Chagas disease. Our results showed that patients with the IND clinical form present significantly higher frequency of Treg cells. Also our data showed that these cells correlate with better heart function (higher LVEF and lower LVDD) in IND patients with chronic Chagas disease. Besides, these patients present significantly higher frequency of Treg IL-10^{+} and IL-17^{+} cells when compared with non-infected individuals and CARD group. Moreover, CARD patients present significantly higher frequency of Treg IL-6^{+}, IFN-γ^{+}, TNF-α^{+} and CTLA-4^{+} cells when compared with IND group. Additionally, Treg cells can suppress the proliferative response in IND patients, although the mechanism is not IL-10 or CTLA-4 dependent. We also demonstrated that effector T cells from IND patients in the presence of Treg cells produce high levels of IFN-γ but also high levels of IL-10. On the other hand, cultures of effector T cells in the presence of Treg cells from CARD group produce high levels of IFN-γ and TNF-α, but no significant amount of IL-10 cytokine. Taken together our data suggest that patients with the IND clinical form of Chagas disease present higher percentage of Treg cells. These cells have an important immunoregulatory role that leads to maintenance of better cardiac function in these patients, probably controlling the exacerbated immune response throughout the modulation of the cytokine environment and/or killing effector cells.

1595

ENHANCED PROTECTION OF MICE UNDERGOING TREATMENT FOLLOW BY REINFECTION WITH *TRYPANOSOMA CRUZI*

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We have previously shown that *Trypanosoma cruzi*-specific CD8+ T cells from mice cured with benznidazole (BZ) expressed high levels of the memory markers CD62L and CD127 and can transfer a degree of protection to high dose challenge infection. Here, we evaluated T cell responses and resistance following repeated treatment and cure. C57BL/6J mice were submitted to one or more rounds of infection with *T. cruzi* and then cured with BZ followed by a final reinfection with *T. cruzi*. For mice receiving a tertiary or quaternary challenge, each foot pad was injected with *T. cruzi* expressing the tdTomato protein and the fluorescence intensity (photons/cm2/sec) was monitored as a measure of parasite load. All groups were compared to mice with persistent primary infection with *T. cruzi* (untreated). Parasite-specific CD8+ T cells from cured mice expanded substantially more upon a rechallenge as compared with the parasite-specific CD8+ T cells from persistently infected/rechallenged mice. Additionally, persistently infected mice can clear a secondary challenge in the site of infection faster than their BZ-treated/cured counterparts. However, cured mice submitted to two or three rounds of treatment follow by reinfection acquired an enhance protection after a tertiary or quaternary challenge as compare with mice receiving only a single round of infection and curative treatment. In addition, *T. cruzi* -specific CD8+ T cells from cured mice undergoing three rounds of reinfection and treatment reexpressed CD127 more rapidly and in higher frequency after rechallenge, suggesting that these mice better regulate parasite load upon rechallange, relative to their untreated/persistently infected counterparts. These results demonstrate that repeated infection and cure fails to induce sterile resistance to infection, calling into question the ability of more conventional vaccination protocols to prevent establishment of infection.

1596

DYNAMICS OF LONGITUDINAL KENYAN INFANT ANTIMALARIA ANTIBODY RESPONSES

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Maternal antibodies transferred to the fetus during pregnancy protect the infant from malaria infection. These antibodies are thought to wane by 6-9 months of age. Infants slowly acquire antimalaria antibodies with repeated infections. Antimalaria antibodies were measured in plasma samples collected from a longitudinal cohort of infants born 2007-2009 (Msambweni, Kenya) with blood samples drawn approximately every

6 months from birth to 36 months of age. Plasma samples from 89 infants (~4.3 blood samples/infant) were examined for the presence and magnitude of multiple antimalaria antibodies by a) serology to 13 malaria antigens, b) human antibody recognition of *Plasmodium falciparum* proteins exported to the erythrocyte membrane from 3 different parasite strains and c) functional antibody-mediated growth inhibition of cultured parasites. We found that, by serology, antibodies directed against the majority of antigens tested (MSP1, EBA175, EBA181, EBA140, LSA, PfCSP, PfCelTos, Sera5) waned by 4-8 months of age. Antibodies directed against AMA1, however, did not wane until 16-23 months of age. For the majority of the antigens tested, infant antibody levels and prevalence reached or exceeded birth levels by 28-32 months of age. In contrast, antibodies directed against *P. falciparum* proteins exported to the erythrocyte membrane waned by 4-8 months and stayed at low levels throughout infancy. Growth inhibitory antibodies waned by 4-8 months of age and were acquired at low rates throughout infancy. These findings call into question the generally accepted paradigm that antibodies directed against *P. falciparum* proteins exported to the erythrocyte membrane (thought to protect against severe malaria) are acquired rapidly upon infection whereas antibodies directed against merozoite surface proteins are acquired gradually and after repeated infections.

1597

DECIPHERING THE MOVING-JUNCTION AMA1-RON2 COMPLEX IN *APICOMPLEXA* PARASITES

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The Apicomplexa phylum comprises a wide range of obligate intracellular parasites, including *Plasmodium spp*. and *Toxoplasm*a, responsible for malaria and toxoplasmosis respectively. These protozoan parasites actively invade their host cells and this process requires the coordinated secretion of specialized secretory organelles, the micronemes and the rhoptries. At an early stage of invasion, a close contact between the parasite and the host plasma membranes, referred as the moving-junction (MJ), act as an anchor onto which the parasite relies on to propel itself forward into the cell. The micronemal protein AMA1 and the rhoptry neck proteins RON2/4/5 secreted into the host cell are the MJ molecular actors identified so far. We have recently demonstrated a direct interaction between AMA1 displayed on the parasite surface and RON2 embedded in the host plasma membrane in *Toxoplasma* and *P. falciparu*m. This binding was shown to be essential for efficient invasion of both parasites. In this study, we have refined the RON2 interacting domain to a short segment located between the two predicted C-terminal transmembrane domains. A RON2 synthetic peptide corresponding to this region (RON2sp) was co-crystallized with the AMA1 ectodomain of *Toxoplasma* and *Plasmodium*. A structure/function analysis led to the identification of key residues essential for the RON2-AMA1 interaction and highlighted the separate co-evolution of the two partners within the phylum. Overall, our results shed light for rationale new drug design targeting the invasion process of Apicomplexa parasites.

1598

CONTINUOUS *IN VITRO* CULTURE OF *PLASMODIUM KNOWLESI* IN HUMAN ERYTHROCYTES: A GENETICALLY TRACTABLE HUMAN MALARIAL PATHOGEN

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Now widely considered the fifth human malaria parasite, *P. knowlesi* has long proven an important model system for malaria, in particular in the study of red blood cell invasion. This is due to the relatively large dimensions of *P. knowlesi* merozoites and their long invasive half-life. Previous work has demonstrated that *P. knowlesi* cultured *in vitro* in macaque erythrocytes is highly amenable to genetic modification, including gene targeting using double-crossover recombination which is relatively difficult to achieve in *P. falciparum*. However, long term culture of *P. knowlesi* in human cells has not been possible, limiting its use to laboratories with access to primate facilities. We have now adapted a laboratory strain of *P. knowlesi* to continuous culture in human red blood cells. Cultures readily reach high parasitaemia(> 10%), display the expected ~24h replication cycle, and can be synchronised, manipulated and cloned as easily as culture-adapted *P. falciparum*. Comparison of the original and culture-adapted *P. knowlesi* lines has shown that without adaptation the original parasites invade and survive very poorly in human cells, indicating that genetic or epigenetic changes were required. Current work is focused on comparing adapted and non-adapted lines to identify key changes required for growth in human erythrocytes. As well as facilitating the adaptation of further lines, this knowledge may reveal requirements for infection of humans in the field. The human erythrocyte-adapted parasites have been used to produce transgenic *P. knowlesi* lines and we will report on progress to further develop genetic methods. We anticipate that human erythrocyte culture-adapted *P. knowlesi* will provide a flexible and genetically amenable model to study many aspects of malaria parasite biology.

1599

GIARDIA FLAGELLAR MOTILITY IS NOT DIRECTLY REQUIRED TO MAINTAIN ATTACHMENT TO SURFACES

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Giardia trophozoites attach to the intestinal microvilli (or inert surfaces) using an undefined suction-based mechanism, and remain attached during cell division to avoid peristalsis. The ventral flagella, one of four pair of motile flagella, are thought to be responsible for generating a hydrodynamic force that translates to a pressure differential, and hence suction, under the adjacent ventral disc. We defined four distinct stages of attachment using TIRF microscopy, imaging structures of the cell body that contact the substrate during attachment. The lateral crest of the ventral disc forms a continuous perimeter seal with the substrate, a cytological indication that trophozoites are fully attached. We then assessed whether the ventral (or any) flagella are necessary for attachment by using TIRF and biophysical assays to quantify attachment strength. If the hydrodynamic model were correct, both strains with defects in flagellar beating should have been prevented from generating a hydrodynamic current, thereby preventing suction. Following a morpholino-based knockdown of central pair protein PF16, both the beating and morphology of flagella were defective, but trophozoites could still initiate proper surface contacts, resisting detachment under both

normal and shear forces. While trophozoites were able to attach like wild type cells, the overall rate of attachment was slower. Over-expression of a dominant negative a2-annexin::GFP (D122A, D275A) resulted in a strain with defects in the ventral flagellar waveform. This strain was also able to initiate attachment comparable to wild type, with only a slight decrease in the ability to withstand normal and shear forces. Thus, when flagellar beating is defective, the positioning and orientation of trophozoites are hindered; however, there is little or no effect on the ability to maintain attachment as evidenced by a continuous ventral disc seal and continuous ventrolateral flange contact.

1600

INROADS OF CALCIUM: HOW EXTRACELLULAR CALCIUM ENTERS AND ENHANCES INVASION-RELATED TRAITS OF THE APICOMPLEXAN PARASITE, *TOXOPLASMA GONDII*

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The apicomplexan parasite, *Toxoplasma gondii,* is a highly successful intracellular parasite capable of invading any nucleated mammalian cell. During the lytic cycle, calcium is a critical element required for motility, invasion, and egress. The importance of intracellular calcium for these traits has been established. However, little is known regarding the role of extracellular calcium. Using the fluorescent, cell permeable calcium indicator, Fura2-AM, we show that tachyzoites of *T. gondii* possess distinct pathways of extracellular calcium entry. This data was further confirmed with manganese quenching experiments. Specifically, tachyzoites have a store-operated calcium entry pathway (SOCE), in which the release of calcium from intracellular stores prompts the influx of extracellular calcium into the cytosol. Extracellular calcium entry was quantitatively inhibited by the calcium channel blocker nifedipine. We assessed the influence of extracellular calcium on invasion-related traits and found that extracellular calcium had a significant influence on microneme secretion. Conoid extension and gliding activity (traits associated with invasion efficiency) increased significantly in the context of store-operated calcium entry. Importantly, blockage of extracellular calcium with nifedipine resulted in a 75% decrease in invasion. These results support a coordinated entry path of calcium from the extracellular environment by which invasion-linked traits can be enhanced and intracellular stores can be replenished. We are currently characterizing calcium entry pathways using two-electrode cell clamping of *Xenopus* oocytes expressing *T. gondii* membrane proteins. This electrophysiological characterization of *T. gondii* ionic channels, the first to our knowledge, will be instrumental in a full understanding of calcium permeation pathways in this ubiquitous model parasite.

1601

BEYOND JUST COUNTING KS AND NS; HIGH THROUGHPUT IMAGE ANALYSIS OF TRYPANOSOMATID CELL ORGANIZATION

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The precise morphology and replication of trypanosomatids facilitates an approach using automated morphometric analysis for investigation of their cell cycle, life cycle stage differentiation, mutants and chemical insults. High throughput image analysis has the capability to accelerate morphological measurement and extract more quantitative data from micrographs. However image analysis of trypanosomatid morphology is complicated by the presence of two, often closely apposed, DNA containing organelles; the kinetoplast and the nucleus. Accurate identification and analysis of these organelles is central to determining cell cycle stage of a cell which is in turn key for understanding the aberrations caused by a drug or mutation. We addressed the difficulties of automated identification of kinetoplasts and nuclei by taking advantage of the different sequence binding biases of different fluorescent DNA stains. We have successfully used colour deconvolution to separate the signal from kinetoplast and nuclear DNA in fluorescence microscopy images. This produces two new images of the same field of cells, one with only kinetoplasts and one with only nuclei. These images are amenable to automated analysis and can also simplify manual analysis of complex phenotypes where kinetoplast and nuclear complement and structure are perturbed. Using our approach to staining, image processing and automated analysis we can analyse around 20000 cells per hour. We have correlated these automated approaches with manual analysis of Trypanosoma brucei and Leishmania mexicana revealing distinct advantages in speed and precision.

1602

NOVEL DRUG UPTAKE AND POTENTIAL RESISTANCE MECHANISMS IN AFRICAN TRYPANOSOMES: SURAMIN

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A better understanding of drug uptake and potential mechanisms of clinical resistance will facilitate the design, application and assessment of more effective therapies. We have carried out loss-of-function screens for drug resistance mechanisms in African trypansomes; all five drugs in clinical use against Human African Trypanosomiasis have been screened using genome-scale RNA interference libraries. The approach was validated by the identification of the nitro pro-drug activator, NTR, and the eflornithine transporter, AAT6 (1). Several novel genes have now been identified, and characterization of suramin uptake will be described; although suramin treatment failures have been reported, no molecular mechanism of clinical or experimental resistance has been documented. Suramin uptake appears to be via receptor mediated endocytosis, and data will be presented to demonstrate roles for an invariant surface glycoprotein, an endosomal membrane channel and the ubiquitin pathway. (1) Baker, Alsford & Horn (2011) Genome-wide RNAi screens in African trypanosomes identify the nifurtimox activator NTR and the eflornithine transporter AAT6. Molecular & Biochemical Parasitology **176** 55-57.

1603

THE CLASS II HISTONE DEACETYLASE PFHDA2 IS AN ESSENTIAL REGULATOR OF *PLASMODIUM FALCIPARUM* VIRULENCE AND GROWTH

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Transcriptional control is essential for the survival of the human malaria parasite *Plasmodium falciparum*. In addition to the unique cascade of gene expression that characterizes normal asexual growth, parasite virulence relies upon regulated expression and silencing within multigene families. This balances the parasite's needs for antigenic variation and immune evasion with the adhesive diversity required for host cell invasion and cytoadherence. Despite the importance of this process, the associated regulatory enzymes remain incompletely characterized. We have identified a protein containing a class II histone deacetylase (HDAC) domain, PfHda2, which plays a critical role in *P. falciparum* transcriptional control. PfHda2 also contains a C-terminal inositol polyphosphate multikinase (IPMK) domain that is conserved among apicomplexan parasites. Protein is detectable in trophozoite and schizont stage parasites and localizes to distinct foci in the nuclear periphery, which in *P. falciparum* preferentially contains clonally variant virulence gene loci. Because multiple attempts

at genetic disruption of the *PfHda2* locus were unsuccessful, we targeted PfHda2 for inducible degradation using a destabilization domain (DD) tag. We observe a >95% reduction of PfHda2 protein in knockdown parasites, leading to elongation of the asexual cell cycle and defective parasite proliferation. As we hypothesized from residence of the protein within the nuclear periphery, PfHda2 knockdown also leads to loss of *var* gene transcriptional repression. This effect is completely independent from the previously observed cell cycle-related defects. These two distinct phenotypes highlight the unique role of PfHda2 as a bi-functional regulator of parasite growth and virulence.

1604

GENETIC ANALYSIS OF *PLASMODIUM FALCIPARUM* GAMETOCYTOGENESIS

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Within the mammalian host, the *Plasmodium falciparum* parasite has two developmental fates: cyclic asexual replication or terminal sexual differentiation (gametocytogenesis). The sexual forms of the parasite (gametocytes) are the only form that is able to survive and propagate in the mosquito vector. Therefore, gametocytes are absolutely essential for parasite transmission. Very little is known about the mechanisms involved in the commitment of *Plasmodium* to sexual differentiation. To gain insight into these mechanisms, we conducted *piggyBac* transposon-mediated insertional mutagenesis screened for parasites that were no longer able to form mature gametocytes. Of 736 parasites (clones) screened in 3 independent transfection experiments, 29 clones did not form gametocytes. We call these clone, insertional-gametocyte mutants (IGMs). For each IGM, insertion of *piggyBac* was verified by Southern blot analysis and the disrupted genes were identified by inverse PCR. This led to the identification of 16 putative gametocytogenesis-disrupting genes. Genetic complementation for 4 of the 16 genes was successfully carried out showing that these genes are essential for gametocytogenesis. To temporally order the 16 genes, we measured their expression pattern along with the expression pattern of other known gametocyte-specific genes in each of the IGMs using RT-PCR. We found a subset of the genes that are likely to act in the initial commitment of the parasite to gametocytogenesis; another subset likely to act during the initial differentiation after the committed merozoite has invaded a new red blood cell; and a third set likely to act early in gametocyte maturation (transition from a stage I to stage II gametocyte). Thus, we have carried out a comprehensive screen for genes essential to commitment and early differentiation of the *P. falciparum* gametocyte. This line of investigation may lead to novel strategies to reduce parasite transmission and disease burden.

1605

STRAIN-SPECIFIC ACTIVATION OF THE NF-κB PATHWAY BY GRA15, A NOVEL *TOXOPLASMA GONDII* DENSE GRANULE PROTEIN

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Toxoplasma gondii is an obligate intracellular pathogen which can modify its environment through the activation and inhibition of host cell signaling pathways. Many different strains of Toxoplasma exist and these strains vary in their effect on such signaling pathways. We have found that type I, II and III strains of Toxoplasma differ in their activation of the NF-kappaB pathway, and activation of this pathway is mediated by the polymorphic Toxoplasma protein GRA15. Type II strains of Toxoplasma activate both NF-kappaB p65 nuclear translocation and NF-kappaB p65-mediated transcription, whereas type II GRA15KO strains do not. GRA15 is also sufficient to activate NF-kappaB p65 when overexpressed in a type I/III strain or in human cells alone. Studies in knockout cell lines show that GRA15 acts downstream of MyD88 and TRIF and upstream or in a complex with TRAF6 and the IKKs. Activation of NF-kappaB affects cytokine production, particularly IL-12, a very important cytokine in Toxoplasma infection, and macrophages infected with a type II GRA15KO strain secrete significantly less IL-12 than macrophages infected with a type II strain. GRA15 also affects cytokine production and parasite growth in vivo. GRA15 is a dense granule protein which is secreted into the host cell upon Toxoplasma invasion, representing the first example of a dense granule protein that can modulate a host cell signaling pathway.

1606

IL-27-DEPENDENT PRODUCTION OF IL-10 BY IFN-γ+ TH1 CELLS IS A CRITICAL MECHANISM FOR PROTECTION AGAINST SEVERE IMMUNOPATHOLOGY DURING MALARIA INFECTION

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Infection during malaria is characterized by strong inflammation. The establishment of a precise balance between the pro- and anti-inflammatory responses may be critical to guarantee control of the parasite and survival of the host. Interleukin-10 (IL)-10, a key regulatory cytokine, has been shown to protect mice against pathology elicited during acute *Plasmodium chabaudi chabaudi* AS model of malaria, however, its crucial cellular source still is a matter of debate. Here, we demonstrate that IFN-g$^+$ Th1 cells are the main producers of IL-10 throughout acute infection, and as a consequence, mice bearing specific deletion of *il-10* in T cells fully reproduce the phenotype observed in deficient IL10$^{-/-}$ mice. The IL-10$^+$ IFN-g$^+$ Th1 cells are highly activated, expressing high levels of CD44 and ICOS and low levels of CD127; they also produce more cytokines than the respective single producing cells. Despite the fact that Foxp3$^+$ regulatory CD4 T cells produce IL-10 during acute infection, highly activated IL-10$^+$ IFN-g$^+$ Th1 cells were shown to be the essential and sufficient source of IL-10 to guarantee protection against severe immune-mediated pathology. Finally, in this model of malaria we demonstrate that the generation of protective IL10$^+$ IFN-g$^+$ Th1 cells is dependent on IL-27 signaling, and independent of IL-21.

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