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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.