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RTS,S/AS01 MALARIA VACCINE CANDIDATE PHASE III SAFETY EVALUATION IN AFRICAN INFANTS 6-12 WEEKS OF AGE AT FIRST VACCINATION OVER FOURTEEN MONTHS OF FOLLOW-UP

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The RTS,S/AS01 candidate malaria vaccine is being evaluated in an ongoing Phase III double-blind randomized trial at 11 research centers in 7 African countries (NCT00866619). The trial has enrolled 15,460 children in two age categories, 5-17 months (N=8923) and 6-12 weeks old at first vaccination (N=6537). In October 2011, the results for the first co-primary endpoint were published including safety data for each age category up to 31st May 2011, as reported previously. Here we report the results of safety evaluation when all infants 6-12 weeks of age at first vaccination have been followed-up for 14 months after the first vaccine dose. Infants aged 6-12 weeks whose parents provided informed consent were randomized 2:1 to receive the RTS,S/AS01 candidate malaria vaccine or a comparator vaccine (meningococcal C conjugate vaccine), administered monthly for 3 doses, in coadministration with a DTPwHepB/Hib vaccine and an oral polio vaccine. Access to clinical evaluation and care were facilitated in all study centers. Safety analyses were performed on the intention-to-treat population. The following safety results will be presented: solicited local and general reactogenicity occurring within 7 days post vaccination and unsolicited adverse events (AEs) within 30 days post vaccination in a subset of 200 subjects from each center (N=2200). Rashes and mucocutaneous changes collected within 30 days post vaccination in the same subset (N=2200) will be presented according to the Brighton Collaboration Guidelines. Serious adverse events (SAEs) (all, fatal, related) will be reported for all infants 6-12 weeks old (N=6537) over a 14-month period after the first vaccine dose. Unsolicited AEs and SAEs will be reported classified by MedDRA preferred term. The evaluation of the safety profile of RTS,S/AS01 in coadministration with the standard EPI vaccines will be key in determining whether the candidate vaccine is suitable for implementation in future vaccination programs in sub-Saharan Africa.

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RTS,S/AS01 MALARIA VACCINE CANDIDATE PHASE III EVALUATION: VACCINE EFFICACY AGAINST CLINICAL AND SEVERE MALARIA IN AFRICAN INFANTS 6-12 WEEKS OF AGE AT FIRST VACCINATION OVER ONE YEAR OF FOLLOW-UP

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The protective efficacy of RTS,S/AS01 candidate malaria vaccine is being evaluated in an ongoing multi-center Phase III double-blind randomized trial conducted in 7 African countries (NCT00866619). In October 2011, the result of the first co-primary endpoint in children aged 5-17 months at first vaccination over 12 months of follow-up was published. We now present the result of the second co-primary endpoint assessing vaccine efficacy (VE) against clinical malaria during 12 months post-vaccination in infants enrolled at 6-12 weeks of age. The trial has enrolled 15,460 children in two age categories, 5-17 months (n=8923) and 6-12 weeks old at first vaccination (n=6537). Infants aged 6-12 weeks whose parents provided informed consent were randomized 2:1 to receive the RTS,S/AS01 candidate malaria vaccine or a comparator vaccine (meningococcal C conjugate vaccine), administered monthly for 3 doses, in coadministration with routine childhood vaccines. Clinical malaria episodes were captured by passive case detection. A standardized clinical algorithm for evaluation of sick children was used to identify severe malaria cases in children presenting to clinical facilities. VE against first clinical episodes was evaluated using hazard rate by Cox regression modelling. VE against all episodes was evaluated using incidence rates by negative binomial regression. Relative risk (proportion affected) was used to evaluate VE against severe malaria. The primary analysis was conducted on the according to protocol population; an analysis on the intention to treat population was also performed. Anti-CS antibody titers were measured with a validated ELISA test at enrolment and 1 month post dose 3. VE against first or only episode of clinical malaria, against multiple episodes of clinical malaria and VE against severe malaria will be presented. Anti-CS antibody response at 1 month post dose 3 will be presented. These results will contribute to the ongoing discussion on the potential role of this vaccine in malaria control programs in sub Saharan Africa.

3

HETEROLOGOUS PRIME-BOOST VACCINATION WITH CANDIDATE MALARIA VACCINES CHAD63 ME-TRAP AND MVA.ME-TRAP IS SAFE AND HIGHLY IMMUNOGENIC FOR EFFECTOR T-CELL INDUCTION IN HEALTHY GAMBIAN INFANTS

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As the global community looks forward to the 2015 target for achieving Millennium Development Goal of combating the scourge of malaria, efforts to develop an effective malaria vaccine are being scaled up. Such a vaccine would complement existing control strategies against the devastating effect of malaria, which is responsible for the death of approximately 750,000 people per year, mostly infants and under-five children. We have previously reported an excellent safety and immunogenicity profile of the candidate malaria vaccines, ChAd63 ME-TRAP and MVA ME-TRAP, in Gambian adults and children. This is a follow-up report of interim findings of evaluation of these vaccines in Gambian infants aged 5-12 months and 10 weeks. Twenty-four healthy infants aged 5-12 months were randomized to receive low or high dose ChAd63 prime vaccination followed by MVA boost vaccination eight weeks later and 12 unvaccinated controls were enrolled in parallel. This was followed sequentially by randomization of another 36 infants aged 10 weeks in the same manner. Safety of the vaccines was assessed by the description of adverse events related to vaccination, as ascertained through clinical assessment and biochemical and haematological tests. Immunogenicity was evaluated by interferon-gamma ELISPOT, and intra-cellular cytokine staining and flow cytometry. The mean haemoglobin, WBC, ALT and creatinine at pre and post-vaccination visits in the low-high dose ChAd63/MVA and control groups were within acceptable ranges. Observed adverse events that appeared related to vaccination included fever and induration at injection site but overall, the vaccination regime was very well tolerated. Strong antigen-specific T-cell responses were observed post-MVA vaccination in the 5-12 month and 10 week old infants and similar levels persisted after day 105. Despite the concern that induced T cell immune responses would be weak in very young infants because they are fairly Th2 switched, our findings suggest that heterogonous prime-boost vaccination with ChAd63 ME-TRAP followed by MVA ME-TRAP continues to exhibit satisfactory safety and potent T-cell immunogenicity in infants living in a malaria-endemic area. These findings support assessment of efficacy of this vaccination approach in infants and young children.

4

PHASE 1 WITH SPOROZOITE CHALLENGE TRIAL: AN OPEN-LABEL DOSE-ESCALATION SAFETY, REACTOGENICITY, IMMUNOGENICITY, AND EFFICACY STUDY OF THE VACCINE CANDIDATE *PLASMODIUM FALCIPARUM* MALARIA PROTEIN (FMP012), AN *E. COLI*-EXPRESSED CELL-TRAVERSAL PROTEIN FOR OOKINETES AND SPOROZOITES (*PF*CELTOS), ADMINISTERED INTRAMUSCULARLY WITH GLA-SE ADJUVANT IN HEALTHY, MALARIA-NAÏVE ADULTS

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A highly efficacious malaria vaccine able to prevent morbidity and mortality caused by *Plasmodium falciparum* is key to the malaria eradication effort. A novel pre-erythrocytic antigen termed cell-traversal protein for ookinetes and sporozoites (CelTOS) plays an important role in the traversal of host cells in both mosquitoes and vertebrates. CelTOS has a highly conserved amino acid sequence among *Plasmodium* species and is crucial for infection suggesting an important functional role across all plasmodia. Falciparum malaria protein 12 (FMP012) is a recombinant subunit protein based on the 3D7 clone of *P. falciparum* CelTOS. FMP012 is expressed in and purified from *E. coli* using a unique synthetic gene construct derived from the "codon harmonization" algorithm for optimal folding and expression. Following demonstration of heterologous protection in mice, we embarked on a first-in-human Phase 1 with sporozoite challenge vaccine study of FMP012 formulated with GLA-SE, an adjuvant system based on a synthetic TLR4 agonist in a stable oil-in-water emulsion designed to enhance both the humoral and cell-mediated immune responses, to evaluate the safety of the vaccine candidate. The study was designed to incorporate dose-escalation of both antigen and adjuvant. A total of 30 volunteers were divided into 3 dosage groups with 10 subjects in each group, each receiving 3 intramuscular vaccine injections at defined intervals. A *P. falciparum*-infected mosquito challenge will be performed in 6 infectivity control volunteers and all 3 vaccine groups 28 days following the third immunization. Volunteers who develop parasitemia will be treated as soon as parasites are identified on blood smear. The study design, safety, reactogenicity, immunogenicity against FMP012, and efficacy of the vaccine against sporozoite challenge will be reported.

5

CONSENSUS ON STANDARDIZATION OF CONTROLLED HUMAN MALARIA INFECTION BY MOSQUITO BITE FOR EVALUATION OF CANDIDATE MALARIA VACCINES

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Controlled human malaria infection (CHMI) is increasingly utilised in early clinical testing of candidate malaria vaccines and antimalarial drugs. As demand for clinical trials employing CHMI has increased, the need to harmonise procedures and methods among the different centres conducting CHMI catalysed the creation of a standardised document outlining the design and conduct of CHMI and a second document for the microscopy methods used to determine the patency endpoint. The standardization specifies considerations for screening potential participants, inclusion and exclusion criteria, parasite and mosquito strains used and techniques for infection of mosquitoes prior to challenge, primary and secondary endpoints to be considered, the use of an infectivity control group, procedures on the day of malaria challenge and follow-up visits, and treatment of participants who experience a patent parasitemia. Volunteer safety was the paramount consideration throughout the discussions. As the primary outcome for CHMI is microscopy-confirmed malaria patency, procedures for optimizing malaria diagnosis are delineated to further support standardisation of clinical trials. The process to achieve harmonised standards focused on agreement of the principles underlying best practice, providing flexibility for centres to conform to the agreed principles using locally appropriate procedures.

6

IDENTIFICATION OF NOVEL CD8+ T CELL-RESTRICTED EPITOPES WITHIN *PLASMODIUM FALCIPARUM* CIRCUMSPOROZOITE PROTEIN (CSP)

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Plasmodium falciparum CSP is a leading malaria vaccine candidate antigen that induces protective immunity in humans, and the potential contribution of cell-mediated immunity is under active investigation. Our recent vaccine trial of DNA-primed adult volunteers boosted with human serotype 5 recombinant adenovirus vectors expressing CSP and

AMA1 induced sterile protection in 4/15 volunteers, and in 2 volunteers protection was linked to CD8+ T responses targeting CSP. Therefore, we have decided to map the minimal CD8+ T cell epitopes within CSP. Volunteers were immunized with recombinant adenoviruses expressing CSP, or CSP and AMA1 (1x10e10 particle units each construct). ELISpot responses peaked at 28 days after immunization, with summed responses using 9 CSP peptide pools containing 15mer peptides overlapping by 11 amino acids, ranged from 95 to 2550 sfc/m. We used computerized algorithms (NetMHC software) to predict and rank potential CSP 8-10mer minimal epitopes using class I binding affinities (IC_{50}<500nm) within 15mers contained in the four most active CSP peptide pools matched to the different HLA A and B alleles expressed by these volunteers. Many predicted epitopes were restricted by more than one HLA allele. 15mers were then tested in ELISpot with volunteers immunized with the same volunteers and many 15mers containing predicted minimal epitopes were positive with HLA-matched volunteers. Next, 16 predicted novel epitopes were synthesized and tested in ELISpot, and nine were positive with HLA-matched volunteers, five from the N- and four from the C-terminal regions. Three epitopes were restricted by a single HLA allele, four epitopes by two HLA alleles, one epitope by three HLA alleles, and the restriction of one epitope was not determined. ELISpot depletion studies, and flow cytometry, confirmed that these responses were CD8+ T cells. In our upcoming trials, we will use these Class 1 epitopes in recalling CD8+ T cell responses and also search for additional epitopes. CSP CD8 T cell epitopes linked to protection could be components of broad population coverage multi-epitope vaccine.

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ANTIBODY SUBCLASS AND AVIDITY RESPONSES TO AMA1 VACCINE CANDIDATE FMP2.1/AS02$_A$

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Both antibody avidity and specific IgG subclass antibodies, particularly cytophilic antibodies IgG1 and IgG3, have been associated with protection in studies of naturally acquired immunity to malaria; however, few studies have evaluated vaccine-induced avidity or subclass antibody levels and their relationship to protection against malaria. In a Phase 2 pediatric randomized controlled trial in Bandiagara, Mali, the recombinant apical membrane antigen 1 (AMA1)-based vaccine candidate FMP2.1/AS02$_A$ elicited strain-specific efficacy against clinical malaria. Although total AMA1 antibody level was not significantly associated with protection, we hypothesized that titers of AMA1-specific antibody subclasses, particularly IgG1 and IgG3, and/or antibody avidity may correlate with protection. We measured titers of AMA1-specific IgG1, IgG2, IgG3, and IgG4 and antibody avidity to AMA1 by ELISA (using urea denaturation for avidity assays) in AMA1 vaccine recipients and control subjects at enrollment and at multiple time points following the last of three vaccinations. Preliminary data from a subset of ten AMA1 vaccinees selected irrespective of their protection status and ten endemic control subjects show that, following vaccination, AMA1-specific subclass titers were higher in AMA1 vaccinees than in control subjects (P<0.0001 for all subclasses 30 days after the last vaccination). AMA1 vaccinees also had a higher ratio of cytophilic to non-cytophilic antibodies than control subjects (P=0.01). However, avidity indices were the same in both groups at the time points tested and did not change after immunization or natural exposure to malaria in either group. Results from a larger subset of AMA1 vaccinees and control subjects will be presented, including the relationship of subclass titer to risk of clinical malaria, stratified by age and by cumulative parasite density measured as area-under-the-curve.

8

COMPARISON OF LONG-LASTING INSECTICIDAL NETS VERSUS LONG-LASTING INSECTICIDAL NETS IN COMBINATION WITH INDOOR RESIDUAL HOUSE SPRAYING FOR MALARIA VECTOR CONTROL: RESULTS OF A CLUSTER RANDOMIZED TRIAL

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There is substantial evidence of the effectiveness of Insecticide Treated Nets (ITN) and Indoor Residual house Spraying (IRS) to protect against malaria. We conducted a cluster randomised controlled trial (CRT) in North-West Tanzania to investigate if there is an additional benefit to combining IRS and Long Lasting Insecticidal bed Nets (LLIN) compared to LLINs alone. A universal coverage campaign (UCC) aimed at providing one LLIN to each sleeping space in all 50 villages in the study area was carried out in 2011. Using restricted randomisation to ensure that the study arms were balanced on baseline factors including infection prevalence, 25 villages were allocated to the control arm (LLINs only) and 25 clusters to the intervention arm (LLIN+IRS). Interior walls of houses in the intervention arm were sprayed with the carbamate insecticide bendiocarb (Ficam, Bayer, Germany) in late 2011 to early 2012. Prevalence of *Plasmodium falciparum* (Pf) infections, clinical malaria and anaemia were compared between the two arms via two cross sectional household surveys 2 and 7 months post intervention. For each cluster approximately 90 children 6 months to 14 years old were randomly sampled. Monthly entomology surveys were conducted in 40 clusters using CDC light traps. Preliminary results from the first post-intervention survey show that prevalence of Pf infection in the intervention arm (IRS+LLIN) was 13.6% (95% CI = 8.4-21.4) compared to 23.5% (95% CI = 15.5-33.9) in control villages (LLINs alone), but the evidence for this difference was marginal (odds ratio = 0.51, 95% CI = 0.24-1.09; p-value=0.08). The range of cluster estimates were 0-48.4% and 0%-75% in the intervention and the control arms respectively. *Anopheles* densities were 82% (p=0.056), 98% (p<0.001) and 79% (p=0.21) lower in the intervention arm compared to the control arm in each of the three months following the intervention. Final results from both post intervention surveys will be presented. Early results from this CRT suggest that using IRS combined with LLINs is likely to be beneficial compared to using LLINs alone.

9

ALL CAUSE UNDER-FIVE MORTALITY DECLINES IN NORTH A AND MICHEWENI DISTRICTS OF ZANZIBAR ARCHIPELAGO, 1999-2008

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Malaria control in Zanzibar archipelago was renewed with the introduction of artemisinin-based combination therapy for uncomplicated malaria in late 2003 and long-lasting insecticidal nets (ITN) and indoor residual spraying in 2006. We assessed all cause under-five mortality (ACCM) in

North A (Unguja Island) and Micheweni (Pemba Island) districts in the pre-intervention (1999-2003) and post-intervention period (1/1/2004-6/30/2008) using complete birth histories of women of reproductive age (15-49 years), obtained from cross-sectional household surveys conducted in May, 2008. Cox proportional hazards models (SAS 9.2 SURVEYPHREG procedure) was used to compute hazard ratios (HR) for deaths in two cohorts of children born during the pre- and post-intervention periods. Rainfall and household ITN ownership were included as time dependent variables in the model. Sandwich variance estimators were used to account for intracluster dependence of variance and associated 95% confidence intervals (CI) and probability values (p) were calculated. In North A, ACCM declined by 17.1% (95% CI, 8.0-33.5, p=0.04) from 86.0 per 1,000 births (95% CI, 73.5-98.6), estimated from 2,184 reported births in the pre-intervention period, to 68.9 per 1,000 (95% CI, 57.0-80.8), estimated from 2,172 reported births during the post-intervention period. In Micheweni, ACCM declined by 41.4% (95% CI 26.3-56.4, p<0.001) from 99.6 per 1,000 births (95% CI, 87.4-111.7), estimated from 2,828 reported births in the pre-intervention period, to 58.2 per 1,000 (95% CI, 48.9-67.6), estimated from 2,961 reported births during the post-intervention period. Adjusting for household wealth quintile, rainfall and household ownership of ITNs, the hazard of ACCM was 45% lower in the post-intervention period (HR=0.55, 95% CI 0.30-0.85, p<0.001) and 41% lower in North A than Micheweni (HR=0.59, 95% CI 0.35-0.94, p<0.001). In conclusion, ACCM markedly declined during the post-intervention period in both districts but the hazard of ACCM was significantly different between the two districts.

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IMPACT OF ANTIMALARIAL INTERVENTIONS ON TRENDS IN MALARIA CASES, HOSPITAL ADMISSIONS AND DEATHS, 2000-2010, RWANDA

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To evaluate the effectiveness of control methods for malaria, the Rwandan government and its partners distributed insecticide treated nets (ITN) and made artemisinin-based combination therapy (ACT) widely available from 2005 onwards. The impact of these interventions on malaria cases, admissions and deaths was assessed at district hospitals. District records of ITNs and ACTs distribution were reviewed. Malaria and non-malaria indictors in 30 district hospitals were ascertained from surveillance records. Trends in cases, admissions and deaths for 2000 to 2010 were assessed by segmented log-linear regression, adjusting the effect size for time trends during the pre-intervention period, 2000-2005. The proportion of the population protected by ITNs increased from nearly zero in 2005 to 43% in 2006, 65% in 2010. In all age groups, from the pre-intervention period, confirmed malaria outpatient cases declined by 74%, (95% Confidence Interval [CI], 24-99%), slide positivity rate declined 73%, (CI, 21%-98%), malaria admissions declined 65% (CI, 31%-95%); and malaria deaths declined 55% (CI, 28%-80%). In children <5, malaria admissions fell 67% (CI, 31-95%); malaria deaths 75%, (CI, 27%-93%); and all-cause deaths 34% (CI, 53%-68%). Concurrently, outpatient cases, admissions and deaths due to non-malaria diseases in all age groups either increased or remained unchanged and rainfall and temperature remained favourable for malaria transmission. Malaria cases, admissions and deaths decreased in regions with both higher (East) and lower (North, South, West) baseline rates. In conclusion, greater than 50% decline in malaria hospital cases, admissions and deaths in Rwanda since 2005 followed a marked increase in ITNs coverage and use of ACT. The decline occurred among children and in all ages, and despite increased hospital utilization and suitable conditions for malaria transmission. Decreased malaria deaths likely account for a portion of the decreased child mortality in Rwanda.

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ACCESS AND TARGETING OF MALARIA TREATMENT: ASSESSING POLICY IMPACT OF THE AFFORDABLE MEDICINES FACILITY - MALARIA AND ROLL OUT OF PARASITOLOGICAL DIAGNOSIS IN THREE REGIONS OF TANZANIA

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Artemisinin-based combination therapy (ACT) is the first line antimalarial in most endemic countries, but there are concerns that access is poor, while targeting to patients with parasitemia is also highly inadequate. In Tanzania national implementation is underway of strategies to improve both access and targeting of ACTs. Access is being addressed through the Affordable Medicines Facility - malaria, whereby quality assured ACTs are heavily subsidised in the public and private sectors. Targeting is being addressed through provision of rapid diagnostic tests (RDTs) and enhanced microscopy in public health facilities. To evaluate the impact of these two interventions, we conducted large scale household surveys at baseline and follow up in three regions with varying malaria transmission (Mwanza, Mbeya and Mtwara, where parasite prevalence was 23.8%, 23.0% and 2.1% respectively in 2010). In 2010 and 2012 we visited 80 randomly selected enumeration areas in each region. At baseline 5,428 households and 20,900 people were interviewed (follow up data collection is ongoing). All household members reporting fever in the past 14 days were asked about treatment obtained. Of those with fever at baseline, 29.5% sought care at a drug store/pharmacy, 19.1% at a government health facility, 11.5% at a general retailer, and 11.7% at other sources. In Mbeya the proportions visiting government facilities and drug stores were almost equal, while in Mwanza many more people visited drug stores and in Mtwara government facilities were the most common source. The percentage of fevers treated at government facilities was 35.0% for children <5 years old and 14.6% for over 5s. At baseline only 10.4% of people reporting fever obtained an ACT the same day or next day of fever onset (18.7% of under 5s and 7.7% of over 5s). Only 10.7% of people with fever received a blood test (19.6% in the wealthiest quintile and 4.2% in the poorest quintile). We will compare baseline findings with those from the endline to assess how access and targeting of drugs have been affected by these two key interventions, and to explore factors associated with ACT and diagnostic test use. These findings will allow exploration of the interaction of large scale access and targeting strategies at the community level, across all age groups, in diverse settings in terms of transmission and access to health care.

12

THE SPATIAL DISTRIBUTION OF MALARIA CASES VARIES DEPENDING ON VILLAGE MALARIA PREVALENCE

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In Zambia the current interventions of insecticide-treated mosquito nets, indoor residual spraying and case management with artemisinin

combination therapy are not likely to result in malaria elimination alone. As part of a pilot mass malaria screening and treatment intervention, 10 health facilities in Gwembe and Sinazongwe districts, Southern Province, Zambia were randomly selected to receive a single round of mass malaria screening and treatment preceding the 2012 high malaria transmission season. In December 2011 and January 2012 approximately 50,000 individuals, regardless of symptoms, were tested for malaria parasites by community health workers using ICT Mal Pf rapid diagnostic tests. Individuals testing positive were treated with artemether-lumefantrine, the national first line malaria treatment. The single round of mass malaria screening and treatment will be evaluated using a combination of study designs and analyses: 1) a randomized post-only comparison between intervention and control areas of parasite prevalence in children < 6 years of age measured through an oversampled malaria indicator survey in April 2012; 2) a randomized post-only comparison between intervention and control areas of parasite prevalence in all individuals measured through the first round of the intervention in June 2012; 3) a pre-post comparison of parasite prevalence within intervention areas (follow-up June 2012); and 4) a randomized longitudinal comparison of monthly outpatient laboratory-confirmed malaria cases recorded from health facilities within the 2 districts. Each method of evaluation has limitations including the lack of a baseline in the randomized post-only comparisons, the lack of a counterfactual in the pre-post comparison, and known biases in health facility routine data. Preliminary results will be available in September 2012.

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IMPACT OF HOME MANAGEMENT OF MALARIA AND PNEUMONIA ON HEMOGLOBIN MEAN AND MALARIA INFECTION IN CHILDREN UNDER FIVE YEARS LIVING IN A RURAL AREA OF BURKINA FASO

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Malaria, pneumonia and anemia remain public health problems in malaria endemic areas.Home management of malaria is a key tool of malaria control. Its acceptability and feasibility have been demonstrated by several studies in Africa and it has shown his efficacy in reduction in both morbidity and mortality in children under five. However, there is limited evidence on reduction of malaria transmission intensity and haemoglobin improvement. In order to assess the effect of home management of malaria and pneumonia (HMMP) on hemoglobin and malaria transmission level in children, we carried out this study in a rural and endemic area of Burkina Faso, where we have implemented a HMMP strategy. We conducted cross-sectional surveys at low (LTS) and high (HTS) malaria transmission seasons. From eligible children, after clinical examination, blood smears and hemoglobin were performed. Randomly, 746 children have been enrolled and allocated in three arms (Control (C), Malaria (HMM) and malaria and pneumonia (HMMP)) with respectively 125 children per arm during HTS. During LTS, 124, 124 and 123 children respectively were enrolled in C, HMM and HMMP arms. Hemoglobin mean was significantly higher through malaria transmission seasons (MTS) in HMM arm (10.08 ±1.08 g/dL (LTS) and 9.08 ±1.57 g/dL (HTS)) compared to C arm (9.50 ±1.58 g/dL (LTS) and 8.41 ±1.41 g/dL (HTS)) (P<0.05). However, hemoglobin mean was 9.81±1.28 g/dL (HMMP arm) vs. 9.50 ±1.58 g/dL (C arm) in LTS (P>0.05) and 9.15 ±1.80 g/dL (HMMP arm) vs. 8.41 ±1.41 g/dL (C arm) in HTS (P<0.05). Plasmodic indexes were 51.6%, 40.3% and 30.1% respectively in C, HMM and HMMP arms during the LTS (P=0.003). During HTS, these parameters were 74.4%, 40% and 29.6% respectively in C, HMM and HMMP arms in HTS (P=0.000). Moreover, gametocyte indexes were 22.6%, 19.4% and 7.3% respectively in C, HMM and HMMP arms in LTS (P=0.003). During HTS these were respectively 14.4%, 9.6% and 9.6% (P=0.381).These results suggest that HMMP strategy could contribute broadly to hemoglobin improvement and to malaria elimination.

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USING COMMUNITY-OWNED RESOURCE PERSONS TO PROVIDE EARLY DIAGNOSIS AND TREATMENT AND ESTIMATE MALARIA BURDEN AT COMMUNITY LEVEL IN NORTHEASTERN TANZANIA

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Although early diagnosis and prompt treatment is an important strategy for control of malaria, using fever to initiate presumptive treatment with expensive artemisinin combination therapy is a major challenge in areas with declining burden of malaria. This study was conducted using community-owned resource persons (CORPs) to provide early diagnosis and treatment of malaria and estimate the burden of malaria in north-eastern Tanzania.In 2006, individuals with history of fever within 24 hours or fever (≥37.5°C) at presentation were presumptively treated using sulphadoxine/pyrimethamine. Between 2007 and 2010, individuals aged five years and above with positive rapid diagnostic tests (RDTs) were treated with artemether/Lumefantrine (AL) while under-fives were treated irrespective of RDT results. Reduction in anti-malarial consumption after introduction of RDTs instead presumptive treatment was also estimated. Trends of malaria incidence and slide positivity rates were compared between lowlands and highlands. Of 15,729 cases attended, slide positivity rate was 20.4% and declined by&gt 72.0% from 2008, to &lt 10.0% from 2009 onwards; and the slide positivity rates were similar in lowlands and highlands from 2009 onwards. Malaria incidence declined consistently from 2008 onwards; and the highest incidence of malaria shifted from children aged 10 years to individuals aged 10-19 years from 2009. Cases with fever at presentation declined slightly, but remained at 40.0% in under-fives and &gt 20.0% among individuals aged five years and above. With use of RDTs, cases treated with AL decreased from 58.0% in 2007 to 1.0% in 2010 and 4,875 courses were saved. With basic training, supervision and use of RDTs, CORPs successfully provided early diagnosis and treatment and reduced consumption of anti-malarials. Progressive declining malaria incidence and slide positivity rates observed suggest that all fever cases should be tested with RDTs before treatment. The current remarkable declining malaria suggests that these areas might be moving from control to pre-elimination levels.

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A SHORTER TIME INTERVAL BETWEEN FIRST AND SECOND DENGUE INFECTIONS IS ASSOCIATED WITH PROTECTION FROM CLINICAL ILLNESS IN A PROSPECTIVE SCHOOL-BASED COHORT IN THAILAND

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Despite the strong association between secondary dengue (DENV) infections and dengue hemorrhagic fever (DHF), the majority of secondary infections are in fact asymptomatic or dengue fever (DF). The determinants of the clinical severity of secondary infections remain unclear, though

some studies have suggested a possible titer-dependent and time-dependent role of cross-protective dengue DENV antibodies. Here, we investigate the association between the time interval separating sequential DENV infections and clinical severity and whether, among individuals with the same interval between infections, there were immunological differences that were associated with disease severity. To assess this, we used data from two phases of a prospective cohort study to detect asymptomatic and symptomatic DENV infections in school-children in Kamphaeng Phet, Thailand, conducted from 1998 to 2002 and 2004 to 2007. Children who experienced at least one DENV infection during their enrollment were selected as the population for analysis. 1696 children had at least one DENV infection detected during their enrollment and 268 of these children had two DENV infections detected. A shorter time interval between the first and second DENV infections detected in the cohort was associated with an increased probability of asymptomatic infection. The association was strongest in children who were seronegative for DENV-1 - DENV-4 by hemagglutination inhibition (HI) assay at enrollment (average interval separating sequential infections of 2.6 years for DHF, 1.9 years for DF, and 1.6 years for asymptomatic infections, p=0.01 by exact Wilcoxon rank statistic). In the final model combining time since first observed infection and the magnitude of the antibody response to first infection, the highest probability of being asymptomatic was observed in individuals who experienced their second infection at shorter intervals after the first infection and with a higher titer HI antibody response generated to the first infection. These findings are consistent with a temporal/immunological model of disease risk where cross-reactive antibodies wane from higher-titer, protective levels to lower-titer, detrimental levels. This is the first time that a temporary window of cross-protection following DENV infection has been demonstrated using human infection data since early observations from human challenge studies in the 1940s.

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CORRELATION OF EXPOSURE TO DENGUE AND CHIKUNGUNYA IN CHENNAI, INDIA: RESULTS OF A SEROLOGICAL SURVEY

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Dengue and chikungunya are rapidly expanding vector-borne viruses transmitted by mosquitoes of the genus Aedes. Few epidemiological studies have examined the burden of these infections in South India despite an increase in the number of reported cases of both diseases in recent years, and a high suitability for transmission. We conducted a household based seroprevalence survey among individuals aged 5-40 years in randomly selected spatial locations in Chennai, a city located in the southern Indian state of Tamil Nadu. Previous exposure to dengue and chikungunya was determined using IgG indirect ELISA (Panbio) and IgG ELISA (Novatec), respectively. A total of 1011 individuals, from 438 households in 50 locations of Chennai were enrolled and provided a blood sample. The median age of participants was 25 years (IQR 15 - 33 years) and 55% were female. We found that 19% (95%CI 17-22%) of participants had evidence of exposure to dengue virus, 44% (95%CI 37-50%) to chikungunya and 10% (95%CI 8%-12%) to both viruses. This is significantly higher than the proportion of individuals that reported having had dengue (1%, 95%CI 0.5%-2%) or chikungunya (18% 95%CI 14%-22%) in the past. Preliminary analyses suggest that a main predictor of prior exposure to dengue was chikungunya seropositivity (OR 1.54, 95%CI 1.07-2.21) and vice-versa, a major predictor of prior exposure to chikungunya was dengue seropositivity (OR 1.51, 95%CI 1.08-2.11), even after adjusting for individual and household characteristics. This is expected given that *Aedes aegypti* is the main vector involved in the transmission of both diseases in Chennai. Chikungunya seroprevalence showed great spatial heterogeneity (ICC 0.20, 95%CI 0.11-0.33, adjusted) and was significantly associated with low income, low educational attainment, shared toilet facilities and lack of access to piped water. Interestingly, dengue seropositivity was not spatially heterogeneous and was not significantly associated with any of these household characteristics. These preliminary results suggest that, in spite of suitable environmental conditions, exposure to dengue in Chennai might be taking place in locations other than the household. We use this data to estimate transmission intensity of both pathogens in this population and explore the association of other individual characteristics (e.g., occupation, daily movement patterns) with seropositivity to dengue and chikungunya.

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FACTORS UNDERLYING SPATIAL DIFFUSION OF DENGUE VIRUSES IN THE AMERICAS

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Phylogeographic methods to infer past patterns of spatial diffusion of genetic lineages are increasingly being applied in infectious disease epidemiology in order to infer the origins and geographic spread of emerging pathogens, and viruses in particular. A Bayesian coalescent phylogeographic approach is particularly attractive in this regard as it provides a rigorous and flexible statistical framework for testing hypotheses about the mechanisms underlying spatial diffusion patterns, while taking into account phylogenetic uncertainty arising from both the sequence data and spatial diffusion process. In this framework, different scenarios and models of spatial spread may be investigated and compared by specifying prior distributions for the diffusion rates among sampling locations. In an attempt to understand factors underlying the spread of Dengue virus (DENV) serotypes 1 - 4 in the Americas, we compared various models of spatial diffusion using two hypothesis testing approaches: a standard approach where each scenario was tested separately on a given data set, and a generalized linear model (GLM) approach where the support for each scenario and its contribution is estimated simultaneously on a data set. In each case, we compared various models that (i) assume equal rates of virus movement between all locations, (ii) more intense virus movement between nearby locations, (iii) accommodate the effect of human population size at the locations under consideration and (iv) incorporate the effects of human movement between locations. Both the standard and GLM approach identified distance between locations as the most significant factor in determining the diffusion patterns observed. However, the GLM approach was more robust in terms statistical support. Based on this approach we found that for DENV-1, -2 and -4, the population size of the donor country and the distance between these countries were the most important factors. For DENV-3, population size of the recipient country was also a contributing factor.

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RECIPROCAL HUMAN MOVEMENT AMONG COMMON PLACES SHAPES THE SPREAD OF DENGUE VIRUS

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For many infectious diseases, human mobility is a fundamental driver of pathogen transmission because it defines when and where susceptible hosts come into contact with pathogen. On a collective level, how movements overlap in space and time will thus determine how pathogen spreads through a population. We used contact-cluster investigations to assess the role of human movement in the transmission of mosquito-borne dengue virus (DENV) in Iquitos, Peru. Residents of households recently visited by febrile individuals were invited to participate in interviews and serological testing for DENV infection. We conducted 124 contact-clusters over 3 transmission seasons, involving 1596 participants. Building on work previously reported, here we show that the proportion of houses infested with DENV (>=1 acute infection) was markedly elevated in contact-clusters initiated by DENV cases compared to DENV-negative controls (0.41 ± 0.05 vs 0.17 ± 0.037, seasons 1 and 2), consistent with the idea that transmission occurs within networks of people connected by reciprocal movement among common places--such as the houses of friends and family. Interviews of contact-cluster participants showed, indeed, that multiple individuals visited the same houses frequently (μ = 8 overlapping movements per contact-cluster). Using an individual-based transmission model, we found that if movements are largely random, there should be no difference in transmission rates between DENV+ and DENV- clusters. As movements become more reciprocal, overlapping at fewer locations, transmission rates diverged and the patterns observed in contact-clusters were recovered. Thus reciprocal movements drive heterogeneity in transmission at fine spatial scales, a result that was robust to the influence of mosquito dispersal in additional simulations. Importantly, this heterogeneity is not observable from assessment of aggregate transmission dynamics. Our findings have significant implications for modeling DENV and other infectious diseases. Surveillance and disease prevention efforts designed with knowledge of the role of reciprocal movements could be substantially more efficient and effective than existing approaches.

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ENHANCED POPULATION-BASED SURVEILLANCE FOR SYMPTOMATIC DENGUE INFECTION IN SALVADOR, BRAZIL

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Dengue has evolved into a major public health problem in Brazil; however, because of its nonspecific clinical presentation, the actual number of annual cases is unclear. In order to better describe the burden of disease and to assess the accuracy of the Brazilian Ministry of Health (MS) clinical diagnostic criteria for dengue (fever plus ≥2 of the following: headache, prostration, retro-orbital pain, myalgia, arthralgia, and rash), a prospective, population-based surveillance study was conducted between April 2009 and March 2011 among people seeking emergency care in an urban slum community in the outskirts of Salvador, Brazil. Patients ≥5 years who were febrile (axillary temperature >37.5°C or reported a history of fever within the 14 days prior to presentation) were invited to participate. Acute and convalescent blood samples were drawn and underwent viral detection and serologic testing for dengue. Of the 3075 community residents evaluated for fever, 578 (18.7%) had laboratory-confirmed dengue infection, with a median age among cases of 19 years. The highest incidence was seen in the 5-14 year old age group (4082/100000 person-years, 95% CI: 3835-4340), compared to 1637/100000 overall. Among patients with dengue, 84% reported headache, 82% had prostration, 72% had myalgias, 50% had retro-orbital pain, 44% had arthralgias, and 21% reported rash. The MS clinical criteria had high sensitivity (91%), but low specificity (14%), and a positive predictive value of 26%. There were no significant differences in diagnostic accuracy based on day of illness, but the criteria had lower sensitivity (86%) and higher specificity (23%) in the youngest age group (5-14 years). Though sensitivity is high, prior analysis has shown that dengue is underreported in this community. In addition, the low specificity of the MS criteria likely means that other common febrile illnesses are being misclassified as dengue. This study highlights the urgent need for improved disease surveillance in poor communities.

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CHARACTERIZATION OF DENGUE FOCI IN HIGHLY ENDEMIC CITIES IN COLOMBIA

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There is evidence that in highly endemic cities, dengue fever (DF) cases persist in certain neighborhoods during inter-epidemic periods. Theory suggests that a pathogen introduced into a community will persist longer if its effective reproductive rate (R) is high. However, neighborhoods will experience dramatically higher rates of dengue fever (DF) introductions during city-wide epidemics. Thus, the proportion of DF cases during inter-epidemic periods may more likely to reflect ecological conditions related to R. Here we present results from the analysis of dengue transmission across 300-500 neighborhoods in each of two highly endemic Colombian cities: Armenia (elevation gradient of 1250-1580 m) and Barranquilla (coastal). For each neighborhood, we obtained 7-10 years of weekly data on probable DF cases, number of houses, housing density, a social class indicator, and altitude. City-wide time series were divided into high (epidemic) and low (persistence) transmission periods. Regression trees were used to build models of two outcomes: total DF cases and the proportion of DF cases in persistence periods. In both cities total DF cases was predicted exclusively by the number of houses. By contrast, elevation and social class were the only predictors of persistence in Armenia and Barranquilla, respectively. Neighborhoods that had more cases than predicted by their number of houses were significantly spatially clustered. In both cities these hotspots were located in areas with intermediate-high persistence indicators, but were also close to the commercial centers of each city. These results suggest that while total DF cases reflect viral introduction through human social networks and are driven by population size, the proportion of cases in inter-epidemic periods reflects R. Hotspots, which combine both high R and social connectivity, may occur in areas that have more cases than predicted by their population size. Assessing spatial variation in the effects of population and the timing of DF cases can streamline dengue control in order to focus resources in areas where virus is maintained or amplified.

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REFINING THE GLOBAL SPATIAL LIMITS OF DENGUE TRANSMISSION IN 2012 BY EVIDENCE-BASED CONSENSUS

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Dengue is a vector borne disease that since its emergence as a public health concern in the 1960s has made a significant contribution to global morbidity, yet the spatial extent of the disease and its burden remain poorly known. Despite independent attempts to map the global distribution of dengue virus transmission there is a lack of consensus on contemporary dengue endemic nations even amongst international health organisations. Here we accumulate the most extensive collection of evidence on dengue distribution published to-date and use a variety of new methods to assimilate this evidence and determine dengue status at the national or sub-national level. Evidence of dengue presence was collected from a variety of sources including published literature, reported case data, news reports, questionnaire responses and Genbank isolates. Further evidence was obtained from a dengue occurrence point database. These diverse sources of evidence were then assimilated by a weighted scoring system that assessed dengue presence or absence on a scale of certainty, or "evidence consensus". The final output was a global map of evidence consensus on national dengue presence in 2012 that identifies important evidence gaps, particularly in Africa and central Asia. Mapping by evidence consensus allows incorporation of multiple evidence types. This offers important advantages over presence/absence mapping and explicitly identifies surveillance needs. The sensitivity of this method also allows us to suggest an urgent review of dengue status in 35 countries previously thought to be dengue-free by the World Health Organization (WHO) or Centers for Disease Control (CDC). This map marks the beginning of a five year study to advance both consensus mapping and disease modelling approaches following expansion of our database through further data acquisition and incorporation of novel evidence categories.

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METABOLIC REGULATION OF UREA SYNTHESIS IN *AEDES AEGYPTI* MOSQUITOES

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In previous reports we have shown that *Aedes aegypti* mosquitoes can use the amide group of glutamine to synthesize uric acid, and then further excrete and metabolize it into allantoin, allantoic acid and urea through an amphibian-like uricolytic pathway. Indeed, mosquitoes have two functional metabolic pathways to synthesize urea: argininolysis and uricolysis. Since both pathways contribute to dispose of nitrogen excess in blood-fed females, we are currently investigating the metabolic regulation of urea synthesis in *A. aegypti*. The expression of arginase (AaA), urate oxidase (AaUO), as well as the expression of both genes simultaneously (AaAUO) were silenced in the fat body (FB) and Malpighian tubules (MT) by RNA interference, and the levels of several nitrogen compounds were quantified in the excreta by mass spectrometry techniques. The expression level of AaA or AaUO in FB and MT is significantly decreased in those mosquitoes injected with dsRNA-A or dsRNA-UO respectively. Surprisingly, the expression of AaA is induced when AaUO is silenced and vice-versa, suggesting the presence of cross-talk regulation between both pathways. In conjunction with these data, at 48 h after a blood meal, the urea levels are not modified in those mosquitoes injected with dsRNA-A or dsRNA-UO. However, a significant increase in allantoin is observed in the excreta of dsRNA-A-injected mosquitoes. The knockdown of AaAUO mainly leads to a decrease in the excretion of urea and allantoin, and an increase in arginine excretion. Interestingly, a temporary delay is observed in the digestion of a blood meal in dsRNA-A, UO or AUO-injected females by Western blotting. Moreover, dsRNA-A-injected females treated with a specific nitric oxide synthase inhibitor show an induction of AaUO expression in FB and MT and a significant increase in the excretion of nitrogen compounds. These results indicate that the urea synthesis in *A. aegypti* is tightly regulated by a complex cross-talking signaling mechanism. The coordinated activity of the pathways involved facilitates the disposal of nitrogen excess in blood-fed mosquitoes.

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MICRORNAS ARE CRITICAL FOR BLOOD DIGESTION AND EGG DEVELOPMENT IN MOSQUITOES *AEDES AEGYPTI* AND *ANOPHELES GAMBIAE*

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Mosquitoes serve as vectors for disease pathogens because they require vertebrate blood for their egg production. Pathogen transmission is linked to repeated cycles of obligatory blood feeding and egg maturation. Understanding of mechanisms governing egg production is necessary to develop novel approaches that limit the spread of mosquito-borne diseases. MicroRNAs (miRNAs) are small non-coding RNAs of about 21–24 nt in length which have been shown to be responsible for post-transcriptional regulation of mRNAs in both plants and animals We have uncovered that in addition to hormones, miRNAs represent essential regulators of blood-meal-activated processes and egg development in female mosquitoes. We have adapted the antagomir technology for *in vivo* depletion of specific miRNAs. Two groups of miRNAs have been distinguished based on their depletion phenotypes in female mosquitoes. Depletion of miRNAs from the first group resulted in severe defects in blood digestion, fluid excretion and egg development. On the contrary, after depletion of miRNAs from the second group female mosquitoes fed and digested blood normally; however, their ovarian development was arrested and they failed to deposit eggs. Moreover, these physiological requirements of miRNAs were conserved in *Aedes aegypti* and *Anopheles gambiae* mosquitoes. Bioinformatics, transgenic and molecular biological approaches are being used for characterization of miRNA gene targets, Thus, utilization of *in vivo* antagomir depletions of specific miRNAs have clearly demonstrated that miRNAs were indispensable for blood digestion and egg development in female mosquitoes.

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STRUCTURAL DETERMINANTS OF *ANOPHELES GAMBIAE*-SELECTIVITY FOR ACETYLCHOLINESTERASE INHIBITOR MOSQUITOCIDES

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To address the growing problem of resistance of *Anopheles gambiae* to pyrethroids, we seek to develop new anticholinesterase mosquitocides that exhibit high selectivity for mosquito vs. human acetylcholinesterase (AChE). Such highly selective inhibitors might be predisposed for low mammalian toxicity, and suitable for deployment via insecticide-treated bednets (ITNs). We have developed several acetylcholinesterase inhibitors that exhibit 100- to 500-fold target site selectivity. In this presentation we will review the structure activity relationships of this class of carbamate inhibitors, and describe a 3D QSAR model of inhibition selectivity. We will also review ongoing efforts to determine which amino acid substitutions in *An. gambiae* AChE relative to human AChE confer the high selectivity seen for selected inhibitors.

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MOSQUITO DEFENSE AGAINST OXIDATIVE STRESS ASSOCIATED WITH BLOOD MEAL: CONCERTED ACTION OF HOST AND GUT MICROBES IN *ANOPHELES GAMBIAE*

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The mosquito gut ecosystem accommodates a dynamic microbiota that is essential for various mosquito life traits. In adult stage, blood meals impose a big impact on the structure microbial community. The catabolism of a blood meal results in a large amount of free heme released from hemoglobin. This leads to the generation of radicals and reactive oxygen species (ROS). As an adaptation, mosquitoes have evolved various mechanical and biomedical mechanisms to protect against these toxic molecules. However little is known how gut microbial residents cope with the oxidative stress. In enteric bacteria, OxyR and SoxR are two major regulons controlling response to oxidative stress. Superoxide is sensed by the SoxR, which further activates soxS to transcribe an array of genes, including superoxide dismutase (SOD) that converts the highly toxic superoxide into a less toxic H_2O_2. In parallel, the OxyR senses the H_2O_2 and regulates the activation of major peroxide-degrading enzymes, including catalase, alkylhydroperoxide reductase (AhpC), glutaredoxin (Grx), glutathione reductase, the ferric homeostasis regulator Fur, and the DNA-binding ferritin-like protein Dps. Catalase and AhpC degrade H_2O_2, Grx help maintain protein thiols in their reduced state, whereas Dps and Fur modulates the metal ion environment of the cell to reduce deleterious Fenton chemistry. In this study, we constructed a metagenomic reference of gut microbiota. Both SoxR and OxyR regulons and associated anti-oxidant genes were identified with multiple taxon origins in the gut microbiome. The RNA-seq data revealed the differential expression patterns of both mosquito gut and microbial oxidative stress responsive genes between the sugar fed and blood fed gut. This suggests that in the gut ecosystem the redox homeostasis is maintained by a concerted antioxidant defense from both host and symbiotic microbes.

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EFFECTS OF BLOOD MEAL ON CELLULAR IMMUNITY IN *ANOPHELES GAMBIAE S.S.*

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Malaria is a major health problem with the mosquito *Anopheles gambiae s.s*. serving as the major vector for the protozoan *Plasmodium falciparum.* Understanding the immune responses of the vector are of critical importance for the development of novel vector control strategies. One main aspect of mosquito immunity involves blood cells, or hemocytes, which constitute the cellular arm of the vector's immune system. Hemocytes express important humoral factors such as TEP-1 and LRIM-1 which limit malaria parasite development. Unfortunately, despite the importance of mosquito cellular immunity, little is known about the dynamics of hemocytes in adult mosquitoes and the consequences of their action on parasite transmission. Circulating hemocyte numbers in *An. gambiae* increase sharply and transiently after a blood meal, possibly to prepare for the multiple pathogens mosquitoes encounter in a blood meal. However, molecular mechanisms behind this phenotype have not been determined. Here we analyzed hemocyte proliferation after a blood meal by (1) hemocyte numbers, (2) DNA synthesis assessed by EdU (thymidine analog) incorporation, and (3) cell cycle analysis assessed by flow cytometery. Data from these multiple approaches clearly show hemocyte proliferation after a blood meal. We also determined activation of the Ras-MAPK pathway in hemocytes after a blood meal, and by RNAi we found this activation to be required for blood meal-induced hemocyte proliferation. Lastly, we determined Ras-MAPK signaling to be required for immunity against the rodent malaria parasite *Plasmodium berghei*. This study starts to elucidate mechanisms behind blood meal-induced proliferation of hemocytes in *A. gambiae* and illustrates hemocytes are more complex and plastic than previously reported.

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THE TCCP PROTEIN FROM *ANOPHELES GAMBIAE* IS REQUIRED FOR THE FORMATION OF A TEP1 CONVERTASE REGULATING LYSIS AND MELANIZATION OF MALARIA PARASITES

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The complement factor C3-like protein TEP1 of the *Anopheles gambiae* mosquito, the main vector of human malaria in sub-Saharan Africa, is required for defence against invading malaria parasites. In the mosquito hemolymph, a proteolytically processed form of TEP1, $TEP1_{cut}$, is found in a complex with two putative recognition receptors, LRIM1 and APL1C, which are also essential for the defence against malaria parasites. Here we show that the initial binding of the LRIM1/APL1C/$TEP1_{cut}$ complex on the parasite and bacterial surfaces induces binding of TCCP, a catalytically inactive clip-domain protease. This new complex acts as a surface-bound TEP1 convertase, activating the circulating full-length TEP1 that then rapidly accumulates on the targeted surfaces. The formation of the TEP1 convertase and subsequent accumulation of TEP1 triggers lysis of malaria parasites or activation of an enzymatic cascade that catalyses melanisation of parasites and bacteria. This is the first demonstration of an insect innate immune cascade resembling the vertebrate complement convertase, revealing that core principles of complement pathway activation have been preserved throughout the evolution of animals. Further understanding of the mechanisms regulating the formation of the TEP1 convertase and how human malaria parasites can evade this potent defence reaction could lead to new strategies aiming at eliminating malaria parasites in the mosquito before they are transmitted to humans.

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THE MOLECULAR BASIS FOR GENETIC RESISTANCE OF *ANOPHELES GAMBIAE* TO *PLASMODIUM*: STRUCTURAL ANALYSIS OF TEP1 SUSCEPTIBLE AND RESISTANT ALLELES

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Thioester-containing protein 1 (TEP1) is a central component in the innate immune response of *Anopheles gambiae* to *Plasmodium* infection. Two classes of TEP1 alleles, TEP1*S and TEP1*R, are found in both laboratory strains and wild isolates, related by a greater or lesser susceptibility, respectively to both *P. berghei* and *P. falciparum* infection. We report the crystal structure of full-length TEP1*S1 and compare it to the structure of TEP1*R1. We also report results from biochemical analysis of EP1*S1 and TEP1*R1, which display a qualitative difference in the reactivity of the thioester and interaction with the heterodimeric complex LRIM1/APL1C. Our results suggest that selective pressure on TEP1 has a dual influence on its biochemical properties such that adaptation of mosquitoes to other pathogens may affect their vector competence with respect to *Plasmodium* infection.

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EVALUATING THE EFFECTS OF MASS AZITHROMYCIN DISTRIBUTIONS ON CHILDHOOD GROWTH AND NUTRITION: LESSONS LEARNED FROM TWO PILOT STUDIES

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Mass azithromycin is administered in many developing countries as a treatment for blinding trachoma. In addition to clearing the ocular strains of chlamydia which cause trachoma, this broad-spectrum antibiotic also has efficacy against an array of pathogens causing respiratory infections, diarrhea, and malaria. A relationship between malnutrition and infection has long been recognized. Further, antibiotics are routinely used as growth promoters in animal husbandry. Here we assess whether mass antibiotics distributed for trachoma control have an effect on growth parameters in children in Ethiopia and Niger. As part of two separate clinical trials, one in Ethiopia and one in Niger, we randomized communities to two different treatment strategies: annual or biannual mass azithromycin treatments in Niger, and biannual or biennial mass azithromycin treatments in Ethiopia. We measured the height, weight, and mid-upper arm circumference (MUAC) of 1,426 children aged 6-60 months from 18 communities in Ethiopia and 24 communities in Niger. The prevalence of wasting was lower in the communities that had received more antibiotic: biannually treated communities in Ethiopia versus biennially treated communities (OR 0.38, 95% CI 0.14 to 1.03); and annually treated communities in Niger versus biannually treated villages (OR 0.75, 95% CI 0.46 to 1.23) neither of these differences were statistically significant (p=0.06 and p = 0.26, respectively). In Ethiopia, the prevalence of stunting and underweight were similar in biannually and biennially treated communities. In Niger, stunting and underweight were lower in the biannually treated communities, but differences were not significant. Communities receiving more frequent antibiotic distributions had less wasting than those treated less frequently, though these studies were not able to demonstrate a statistically significant difference. Longitudinal analysis of anthropometric measurements may be required to further characterize the relationship between antibiotic use and childhood growth and nutrition.

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RADIO MESSAGING IN MALI: THE USE OF MASS MEDIA TO PROVIDE INFORMATION ABOUT KNOWLEDGE AND BEHAVIOR CHANGE FOR TRACHOMA ELIMINATION

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Since 2008, the National Blindness Prevention Program in Mali has broadcast messages on the radio about trachoma as part of the country's strategy to eliminate blinding trachoma by 2015. In 2011, a radio impact survey using multi-stage cluster sampling was conducted in the regions of Kayes and Segou to assess radio listening habits, coverage of the messages, knowledge and behavior specific to trachoma prevention, and facial cleanliness of children. Across both regions, a total of 391 adults and 687 children participated in the survey in 16 villages. Both radio access (87.2%) and listening (91.4%) were found to be high, with 59.7% of respondents having heard a message on the radio about trachoma. 78.7% of respondents knew about trachoma, its root causes (64.3%), its impact on sight (86.6%), and disease prevention measures (84.8%). Additionally, 65.5% reported washing their children's faces more than twice/day and 93.8% reported disposal of feces in a latrine. A high percentage of persons who gave a positive response to knowledge and behavior questions reported hearing the trachoma messages on the radio, ranging from 57% to 73% across key responses. 42.4% of respondents reported the radio as their primary source of trachoma information and 14.3% reported the radio along with other sources (ie, health agent, community member, religious leader). There was no difference in ocular or nasal discharge when comparing children whose primary caregiver had/had not heard the trachoma messages. The results from this survey underscore the power of community radio in Mali and its ability to reach populations with important public health information that otherwise would have been difficult and cost-prohibitive to reach. Future plans include a strategy whereby the broadcasting of radio messages will work in tandem with women's groups, community health workers, and community leaders to bring information about trachoma prevention to communities in districts where trachoma interventions are still ongoing, and those that have entered post-endemic surveillance.

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ACCURACY OF RECALL DURING COVERAGE SURVEYS FOLLOWING AN INTEGRATED MASS DRUG ADMINISTRATION

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Mass drug administrations (MDAs) are an effective means of eliminating and controlling neglected tropical diseases. Coverage estimates allow for the evaluation of MDAs and may be generated using distributor records (reported coverage) or by household coverage surveys. Coverage surveys may be used to validate reported coverage; however, they rely on the accuracy of survey respondent recall. This paper describes an evaluation of the accuracy of recall during coverage surveys following an integrated MDA. Independent recorders accompanied drug distributors during the MDA in six villages in Koza district, Cameroon to create a gold-standard drug register. A total of 3,622 individuals, in 535 houses, were registered_independent of eligibility for MDA participation. Individuals were registered with information including description of their house, name, sex, age, if they took each of the three medications (Albendazole, Ivermectin, and Azithromycin) and if so, how many pills they took. Coverage surveys were conducted by independent surveyors at three time points (two, seven and ten months following the MDA). For each surveys, houses were systematically sampled, each time starting with a different house to ensure independent samples. During the surveys, registered persons in selected houses were asked if they took each of the drugs and if so, how many pills. Drugs were described verbally using size, shape and color to differentiate. Surveyors and respondents were blinded to registry responses. Data were entered into Epi Info 6 and analyzed using SAS 9.2. Upon analysis, responses from the gold-standard registry were compared with survey responses. Percent concordance was calculated by determining the proportion of individuals that provided the same answer in the survey as was present in the registry. Preliminary analysis of the two month post-MDA coverage survey showed a 90% response rate (n=1096), and concordance estimates of 76%, 77% and 78% for Albendazole, Ivermectin and Zithromax, respectively. Data collection and analysis are ongoing and will be presented.

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GEOGRAPHICAL FACTORS AFFECTING THE IMPLEMENTATION OF ALTERNATIVE STRATEGIES FOR LYMPHATIC FILARIASIS ELIMINATION IN POST-CONFLICT COUNTRIES

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Vector control, including the use of bednets, is recommended as a possible strategy for eliminating lymphatic filariasis (LF) in post-conflict countries such as the Democratic Republic of Congo (DRC). This study examined the geographical factors that influence community bednet coverage in DRC in order to identify the hard-to-reach areas that need to be better targeted. In particular, urban/rural differences and the influence of population density, proximity to cities and health facilities, plus access to major transport networks were investigated. Demographic and Health Survey geo-referenced cluster data were used to map the proportion of households with at least one bednet (unspecified), with at least one insecticide-treated net (ITN) and ITNs per person for 300 communities. Spatial statistical methods and bivariate and multiple logistic regression analyses were used to determine significant relationships. Overall, bednet (30%) and ITN (9%) coverage were very low with significant differences found between urban and rural communities. In rural communities coverage was significantly positively correlated with population density ($p<0.01$), and negatively with the distance to the two largest cities, Kinshasa or Lubumbashi ($p<0.0001$). Further, coverage was significant negatively correlated with the distance to primary national roads and railways (all bednet measures), distance to the main river (unspecified only) and the distance to the nearest health facility (ITNs only). A logistic regression model fitted to the rural community data indicated that, after controlling for the effects of the measured covariates, coverage levels in the Bas-Congo province close to Kinshasa were much larger than expected. This was most noticeable when considering ITNs and ITNs per person, which were 5.1 times higher in the Bas-Congo province compared to all other provinces. These maps and spatial analyses provide key insights into the barriers of bednet coverage and will help to inform both LF and malaria bednet distribution campaigns as part an integrated vector management strategy.

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MODELING THE IMPACT AND COSTS OF SEMIANNUAL MASS DRUG ADMINISTRATION FOR ACCELERATED ELIMINATION OF LYMPHATIC FILARIASIS

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The Global Programme to Eliminate Lymphatic Filariasis (LF) has a target date of 2020. The Programme is progressing well in many countries. However, progress has been slow in some countries, and others have not yet started their Mass Drug Administration (MDA) programs. Acceleration is needed. We studied how increasing MDA frequency from once to twice per year would affect program duration and costs using computer simulation modeling and cost projections. We used the LYMFASIM simulation model to estimate how many annual or semiannual MDA rounds would be required to eliminate LF for Indian and West African scenarios with varied pre-control endemicity and coverage levels. Results were used to estimate total program costs assuming a target population of 100,000 eligibles, a 3% discount rate, and not counting the costs of donated drugs. A sensitivity analysis was done to investigate the robustness of these results with varied assumptions for key parameters. Model predictions suggested that semiannual MDA will require the same number of MDA rounds to achieve LF elimination as annual MDA in most scenarios. Thus semiannual MDA programs should achieve this goal in half of the time required for annual programs. Due to efficiency gains, total program costs for semiannual MDA programs are projected to be lower than those for annual MDA programs in most scenarios. A sensitivity analysis showed that this conclusion is robust. Thus, semiannual MDA is likely to shorten the time required for LF elimination in countries where it can be implemented. Accelerated MDA provides other benefits including reduced program costs in most situations. This strategy may improve prospects for global elimination of LF by the target year 2020.

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EVALUATION OF THE IMPACT OF MASS DRUG ADMINISTRATIONS (MDAS) USING AN INTEGRATED SENTINEL SITES APPROACH

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Neglected tropical diseases (NTDs) afflict billons of people worldwide resulting in the reduction of both quality of life and workforce capacity. Fortunately, effective drugs are available to help in the elimination and control efforts of five of the most common NTDs through mass drug administrations (MDAs). Current protocols to evaluate the impact of MDAs are disease specific; however, CDC is field-testing an integrated sentinel site approach for evaluating the impact of MDAs for lymphatic filariasis, trachoma, schistosomiasis and soil-transmitted helminths. This abstract focuses on the impact assessment for trachoma although data for all diseases with ongoing MDAs within the districts are included in this project. Prevalence data are being collected yearly for 3 years in four sentinel sites in the Gwadabawa district in Sokoto State, Nigeria and in the health district of Dô in Burkina Faso. Sentinel sites were selected based on NTD prevalence and geographical representativeness. To validate the data, the WHO recommended trachoma prevalence survey is being implemented simultaneously each year with Trachomatous Inflammation - Follicular (TF) as indicator. For each district, 2000 children (250 1-5 year olds and 250 6-9 year olds per site) are surveyed each year in the sentinel sites and 1600 children (80 children per cluster, 1-9 year olds) are surveyed for the trachoma prevalence survey. For year 1 in Nigeria, sentinel site prevalence ranged from 14.3% to 29.8% and the WHO prevalence survey was 16.4% [95% CI: 14.8-18.0]. Prevalence for Year 2 from sentinel sites ranged from 4.8% to 9.3% and the WHO prevalence survey was 10.9% [95% CI: 9.5-12.5]. For Year 1 in Burkina Faso, prevalence from the sentinel sites ranged from 7.9% to15.2% and the WHO prevalence survey was 5.7% [95% CI: 3.8-7.6]. Year 2 data for Burkina Faso and Year 3 data for Nigeria will also be presented. Future data collection will show if the sentinel site approach could provide a simplified yet reliable means of indicating when to conduct surveys to assess whether MDA interventions can be stopped.

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SCHISTOSOMIASIS ELIMINATION IN ZANZIBAR (UNGUJA AND PEMBA ISLANDS): DESIGN AND IMPLEMENTATION OF AN INTEGRATED MULTIDISCIPLINARY PROGRAM

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Elimination of schistosomiasis has been achieved in the past (e.g. Tunisia and Japan), and the WHO has issued a recent call that all countries endemic for schistosomiasis should intensify control interventions and strengthen surveillance, with the ultimate aim to eliminate the disease. On the Zanzibar islands (Unguja and Pemba) offshore of Tanzania, urinary schistosomiasis has been a major public health problem until the mid-1990s. In the meantime, a control program emphasizing preventive chemotherapy with praziquantel significantly reduced the prevalence and intensity of *Schistosoma* haematobium infections. In 2011, the Zanzibar Ministry of Health declared that efforts will be further intensified with the goal of eliminating schistosomiasis from the islands. Therefore praziquantel will be administered to the population every 6 months for at least 2-5 years. A randomized trial focusing on 45 communities on each island will compare snail control and behavior change strategies layered on top of preventive chemotherapy in three study arms, each consisting of 15 communities in Unguja and Pemba. Baseline surveys conducted in early 2012 revealed that 5% of first year students, 4% of children aged 9-12 years and 3% of adults were infected with S. haematobium in Unguja. In Pemba, respective prevalences were 12%, 8% and 6%. Infection hot-spots with prevalences up to 37% were identified. Bulinus globosus, the intermediate host snail, was found in waterbodies in 5 and 10 among the 15 surveyed communities in Unguja and Pemba, respectively. Snail control using niclosamide will start in June 2012. Behavior change interventions developed in collaboration with the community will focus on modifying risky behaviors of children through increased knowledge, encouraging safe play for children, and developing acceptable sanitary facilities. The study will provide an evidence-base for program decisions about schistosomiasis elimination not only in Zanzibar, but also for other settings in Africa that aim to eliminate schistosomiasis.

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MICROFILARIAE OF *BRUGIA MALAYI* INDUCE INFLAMMATORY AND REGULATORY RESPONSES IN DIFFERENT SUBSETS OF HUMAN MONOCYTES

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Monocytes in filarial infections have been shown to modulate filarial-specific T cell responses either through pathways associated with alternative macrophage activation or through the production of IL-10 and possibly indoleamine 2,3 dioxygenase (IDO). Because blood monocytes can be subdivided into three populations based on their surface expression of CD14 and CD16 (CD14hi/CD16- [classical], CD14+hi/CD16med [intermediate], and CD14+/CD16hi [non-classical]) and because the CD14+CD16+ subsets have been shown to be expanded in numerous acute and chronic inflammatory conditions, we chose to explore the ontogeny and function of regulatory monocytes in filarial infection by differentiation of flow-sorted CD14+CD16-, CD14+CD16med and CD14+CD16hi monocytes in response to live microfilariae (mf) of *Brugia malayi* (an alternative and pro-inflammatory activator) and compared this to the response to IL-4 (an alternative activator) or LPS/IFN-γ (classical proinflammatory activator). Using confocal microscopy, our data suggest that the CD14+CD16- subset (in contrast to the intermediate or non-classical monocytes) is the only subset capable of internalizing mf antigens. Moreover, the CD14+CD16- subset is the source of the parasite-driven alternative activation (based on the expression of CD206, CCL13, CCL17, and CCL22) and inflammatory responses (based on the production of IL-6 and TNF-α) as compared to the other two subsets. Interestingly, the regulatory molecules IL-10 and IDO induced by mf are derived from the intermediate monocyte subset. In addition, mf inhibited the function of non-classical (CD16hi) monocytes by diminishing their ability to transmigrate through endothelial monolayers, a process that may relate to the downregulation of CD54 and CD31 cell surface expression; adhesion molecules involved in trans-endothelial migration of monocytes. Our data suggest that the mf induce alternatively activated and regulatory monocytes derived from two distinct human monocyte subsets and influence the function of the third non-classical monocyte subset.

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HUMAN NATURAL REGULATORY T CELLS PRODUCE SOLUBLE FACTORS THAT SUPPRESS EFFECTOR T CELL FUNCTION IN A CONTACT-INDEPENDENT MANNER

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Chronic patent human filarial infections have been shown be associated with a predominant regulatory environment characterized by increased frequencies of both adaptive and natural regulatory T cells through increased production of regulatory cytokines such as IL-10 and TGF-β. Recently we have shown that the nTreg expansion in patent filarial infections could modulate antigen presenting cell (APC) function, but did so indirectly by modulating effector T cell cytokine/chemokine production. To delineate further the mechanisms by which nTregs suppress effector T cell function, we found that, contrary to accepted dogma, nTregs inhibited effector T cells in a manner that was independent of cell contact. Thus, activated nTregs in transwells suppressed autologous effector T cell proliferation by 61%, $p<0.006$) and did so in a dose-dependent manner. Using concentrated supernatant from large numbers of activated nTregs cultured in serum free media that was then subjected to trypsin digestion and mass spectroscopy, the entire nTreg secretome was resolved. Among the most abundant proteins with the potential to mediate the suppressive, contact independent function of nTregs were TGF-β, WISP3 (CCN6) a tumor suppressive gene and PSG1 a suppressive protein that has been shown to induce large production of IL-10 and TGF-β by human monocytes. We found that either alone or in combination recombinant WISP3, PSG1 and TGF-β inhibited (range 42% -87%) effector T cell proliferation in a dose-dependent manner. We are currently assessing the role of each of these molecules in mediating the contact-independent mechanism of suppression by nTregs by RNAi in nTregs and using specific depletion of each of these molecules from nTreg supernatants. Nevertheless, we have clearly identified an important mechanism of effector T cell suppression in human filarial infections.

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A POLYMORPHISM OF *NOD2* GENE ASSOCIATES WITH AUGMENTED FREQUENCY OF TBET+/IL-17+/IFNγ- T LYMPHOCYTES AND SUSCEPTIBILITY TO OCULAR TOXOPLASMOSIS

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Toxoplasmosis is a parasitic infection that has the protozoan *Toxoplasma gondii* as its aetiological agent. In immunocompetent patients, retinochoroiditis (RC) is the most common clinical manifestation of the infection. In this study, we assessed 119 parent/offspring and sibship trios with cases of chronic ocular toxoplasmosis (COT) from Brazil, to evaluate potential associations with polymorphisms in the *NOD2* gene. Three haplotype tagging single nucleotide polymorphisms (tag-SNPs) within the *NOD2* gene were genotyped. The Family Based Association Test (FBAT) showed that the SNP rs3135499 is associated with retinochoroiditis (p=0.039). We then characterized the cellular immune response of 59 cases of COT, four additional cases of active ocular toxoplasmosis (AOT) and the appropriate controls which included: asymptomatic seropositive individuals and uninfected seronegative subjects. We found no differences in cytokines (IFN-γ and IL-2) that are conventionally produced by Th1 lymphocytes when comparing patients with AOT or COT to asymptomatic individuals. Unexpectedly, we found an increased IL-17A production by peripheral blood mononuclear cells from patients with either stage of ocular toxoplasmosis. The higher production of IL-17A also associated with the polymorphism rs3135499 in NOD2 gene. Additionally, the main source of IL-17A was shown to be $CD4^+$/T-bet^-/IFN-γ^-, named Th17 lymphocytes, which were present in higher frequency in patients with scarred or active toxoplasmic retinochoroiditis. Altogether, our results suggest that NOD2 influences the production of IL-17A by $CD4^+$ T helper lymphocytes, which in turn mediates the inflammatory process and might contribute to the development of ocular lesions in patients infected with *T. gondii.*

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INNATE HELPER 2 CELLS IN HUMAN FILARIAL INFECTIONS

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The CD4+ T cell response in patent filarial infections is characterized by an expanded Th2 repertoire at the onset of microfilaremia that gradually contracts with longstanding infection due in large part to the expansion of adaptive regulatory T cells and other sources of IL-10. The factors and cells involved in initiating the Th2 expansion are not fully understood. Recently, a group of innate cells that respond to IL-25 and IL-33 by producing IL-13 and IL-5 have been described. Deemed innate helper type 2 cells (IH2 cells), multi-potent progenitor cells, natural helper cells or nuocytes, these cells were identified in mice and found to be crucial for initiating the immune response to intestinal helminths. Cells with similar phenotypic characteristics have been found in normal human peripheral blood. To examine the role of IH2 cells (Lineage-, CD45+, cKit+ and IL-7Ra+) and nuocytes (lineage-, CD45+, ICOS+, IL-17RB+ and ST2+) in patent filarial infection in humans, we enumerated these cells using multiparameter flow cytometry in 11 relatively acutely infected microfilaremic (MF+) patients (exposure history less than 2 years), those with patent longstanding infection (exposure lifelong), and uninfected controls. Using cryopreserved PBMCs from acute MF+ patients, there was an increased frequency of IH2 cells (GM 0.05 vs 0.02 p=0.05) and nuocytes (GM 0.18 vs 0.06 p=0.31) in MF+ patients as compared to uninfected normal controls. Each of these cell populations produced IL-13 using intracellular staining. In contrast analysis of 11 patients with longstanding patent filarial infection and 15 uninfected subjects showed a small but insignificant increase in the frequency of IH2 cells compared to the normal subjects (GM 13.75 vs. 48.67, p=0.38). To study the function of these IH2 cells more specifically, these have been purified by cell sorting and assessed for their ability to make Th2 cytokines in response to stimulation with IL-2, IL-7, IL-25 and IL-33. Cytokine stimulated highly purified IH2 cells produced large amounts of IL-13, IL-5 and IL-4 compared to cells exposed to media alone. Studies are currently underway to explore the relationship between these IH2 cells and Th2 development in the early (acute) response to filarial nematodes.

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THE CHEMOKINE CXCL12 AND ITS RECEPTOR CXCR4 ARE ESSENTIAL FOR THE CLEARANCE OF THE FILARIA *LITOMOSOIDES SIGMODONTIS* IN RESISTANT MICE

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Litomosoides sigmodontis is a cause of filarial infection in rodents. The outcome of infection is dependent on the parasite's modulatory ability and on the host genetic background. The goal of this study was to determine whether the chemokine axis CXCL12/CXCR4, which notably participates in the control of immune surveillance, can influence the outcome of the infection. We compared *L. sigmodontis* infection of wild type (WT) $Cxcr4^{+/+}$ BALB/c susceptible strain of mice, WT $Cxcr4^{+/+}$ C57BL/6 resistant mice and $Cxcr4^{+/mutant(1013)}$ C57BL/6 mice. On one hand we showed that rapid parasite clearance was associated with a *L. sigmodontis*-specific CXCL12-dependent cell response in WT C57BL/6 mice *vs* WT BALB/c mice and that CXCL12 was produced mainly by pleural mesothelial cells. On the other hand, we evidenced a faster and stronger filarial reduction in $Cxcr4^{+/mutant(1013)}$ C57BL/6 mice *vs* WT C57BL/6 mice, likely due to early defects in infective larvae lymphatic migration. Furthermore, interfering with the CXCL12/CXCR4 axis in both strains of WT mice delayed filarial development, as evidenced by the postponement of the fourth molting process. Moreover, the *in vitro* growth of stage 4 filariae was favored by the addition of low amounts of CXCL12. The CXCL12/CXCR4 axis thus appears to have a dual effect on the *L. sigmodontis* life cycle: by acting as a host-cell restriction factor for infection, and as a growth factor for worms.

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CHARACTERIZING THE IMMUNOPROTEOME OF *VIBRIO CHOLERAE*

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Vibrio cholerae causes an estimated 3-5 million cases and 100,000 deaths annually. Although current vaccines have been shown to be safe and immunogenic, none provide the long-lasting protective immune responses seen with natural infection. Characterization of immunogenic *V. cholerae*

antigens could lead to a better understanding of protective immunity in human cholera infection. Using a high-throughput proteomic-based platform called the Nucleic Acid Programmable Protein Array (NAPPA), we screened 3,761 *V. cholerae* open reading frames (97% of the ORFeome) for anti-*V. cholerae* IgG and IgA responses in 25 cholera patients, 10 vaccinees who received whole cell-killed vaccine with recombinant cholera toxin (WC-rBS), and 10 North American volunteers. We detected significantly higher IgG and IgA reactivity in convalescent sera to a number of previously identified immunogenic and virulence-associated proteins (e.g. cholera toxin B, CtxB; toxin co-regulated pilus A, TcpA; V. cholerae cytolysin, VCC/hlyA) when compared to acute sera, healthy Bangladeshis (pre-vaccine) and/or North American volunteers. We also identified several flagellin proteins including FlaB and FlaC, a number of methyl accepting chemotaxis proteins, general secretion pathway proteins within the Eps operon which have been shown to involved in cholera toxin secretion, and a large number of proteins of unknown function. Of particular interest were those proteins that had detectable IgG and IgA immunoreactivity during natural infection, but not after vaccination, including hemolysin A, TcpA, and FlaC. This study gives insight into differences in immune responses elicited after natural infection and vaccination, and may aid in the development of improved cholera vaccination approaches.

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COMPARISON OF POLYSACCHARIDE ANTIBODY RESPONSES IN CHILDREN RECEIVING TWO DOSES OF A KILLED ORAL CHOLERA VACCINE COMPARED TO RESPONSES FOLLOWING NATURAL CHOLERA INFECTION IN BANGLADESH

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Current oral cholera vaccines induce lower protective efficacy and a shorter duration of protection against cholera than that afforded by wild type infection. This difference is most pronounced in young children. Immunity against cholera is sero-group specific, and while anti-*Vibrio cholerae* lipopolysaccharide (LPS) immune responses are associated with protection against disease, responses against *V. cholerae* O-specific polysaccharide (OSP), the antigen that mediates sero-group specificity, remain to be characterized. Here we report a comparison of polysaccharide immune responses in infants (6-24 months old; n=15), toddlers (3-5 years of age, n=15), and older children (6-14 years of age, n=15) from an endemic region receiving two doses of a killed oral cholera vaccine containing recombinant cholera toxin B subunit 14 days apart. We found that infants are unable to mount IgG, IgA, or IgM antibody response to *V. cholerae* OSP 7 days after a second dose of vaccine, whereas toddlers and older children are able to mount significant ($P<0.05$) and comparable IgG and IgA responses against both OSP and LPS. We also demonstrate that baseline levels of anti-OSP IgM and IgG in toddler vaccinees are significantly higher ($P<0.01$) than the baseline of infants, and comparable to those in older children, suggesting that children in this endemic region likely have repeated antigen exposures at all ages. In comparison to vaccinees, toddlers (n=15), and older children (n=15) with wild type *V. cholerae* O1 Ogawa infection mounted significantly higher ($P<0.05$) IgM and IgA day 30 antibody responses to both LPS and OSP. Our findings demonstrate that infants are unable to mount acute antibody responses to *V. cholerae* polysaccharide antigens following oral cholera vaccination, and that day 30 anti-OSP responses in child vaccinees are appreciably lower than that induced following wild type disease. These findings suggest that targeting of anti-polysaccharide responses may be critical in achieving optimal cholera vaccine efficacy, especially in young children.

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ANTIGEN-SPECIFIC MEMORY T CELL RESPONSES AFTER VACCINATION WITH AN ORAL KILLED CHOLERA VACCINE IN BANGLADESHI CHILDREN AND COMPARISON WITH NATURAL CHOLERA

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Young children, older children and adults develop comparable levels and duration of immunity following cholera. In comparison, young children receiving oral killed cholera vaccine (OCV) develop lower level and shorter duration of protection compared to older children and adults. The reasons for this are unclear. We investigated OCV-induced memory T cell responses in younger and older children, and compared responses to those in children with cholera. We found that patients with cholera developed significant toxin-specific effector memory T cells (TEM) with follicular helper and gut homing characteristics. Older children (6-14 years of age) receiving two doses of OCV containing recombinant cholera toxin B subunit (rCTB) had more modest TEM responses with follicular helper and gut homing characteristics, but younger vaccinees (24-71 months of age) did not develop TEM responses. The TEM response correlated positively with subsequent IgG memory B cell responses specific to rCTB in older vaccinees. Cytokine analyses indicated that cholera patients developed significant Th1, Th17 and Th2 responses, while older children receiving vaccine developed more modest increases in Th1 and Th17 cells. Younger vaccinees had no increase in Th1 cells, a decrease in Th17 cells, and an increase in Treg cells. Our findings suggest that T memory responses are markedly diminished in children receiving OCV, especially young children, compared to responses following wild type cholera, and that these differences affect subsequent development of memory B cell responses. These findings may explain the lower efficacy and shorter duration of protection afforded by OCV in young children.

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EFFECTS OF UNDERNUTRITION AND PARASITIC LOAD ON IMMUNE RESPONSE TO ORAL CHOLERA VACCINE DUKORAL IN BANGLADESHI CHILDREN

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Immunogenicity and efficacy of oral vaccines in children are low in developing countries and may depend on genetic make-up, age, nutritional status, parasitic infestations, maternal antibodies as well as pre-existing microbial load in the gut. A relationship between micronutrient supplementation with vitamin A, zinc and withholding of breast feeding has been found to enhance vaccine specific responses. The role of nutritional status and parasitic infestation on the immune responses to vaccines has not been studied widely. We determined the impact of under nutrition and parasitic load on immune response of the cholera

vaccine Dukoral. After screening of 952 children (2-5 years) 204 children were recruited and had high parasitic load (100 eggs/gm). Children were divided into two groups (n=102×2) where one group of children took the antiparasitic drugs 7 days prior to immunization and the other group took placebo. Anthropometric measurement for the children 'weight for age (WAZ)'and 'height for age (HAZ)'were calculated in relation to the NCHS reference (< -2 Z score used for underweight and stunting). Increases of vibriocidal antibody responses three weeks post vaccination were observed in all vaccine recipients after intake of two doses of the vaccine (P<0.001). No significant differences were antibody titers between children in the treated and non treated group (77% vs 74%). Response rate and magnitude of responses of CTB specific IgA and IgG antibody and LPS specific IgA and IgG antibody responses were also seen in both groups (P<0.001). Of the children 45% were underweight and 55% were stunted. Analyses showed that immune response are significantly lower in malnourished children (underweight, P= 0.036; stunted, P =0.048) than those that were well nourished. Our results suggest that hypo responsiveness to oral vaccination may not arise due to high parasitic load but due to poor nutritional status. This suggests that malnutrition is a major impediment to vaccine responses.

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COMPARISON OF IMMUNE RESPONSES TO THE O-SPECIFIC POLYSACCHARIDE AND LIPOPOLYSACCHARIDE OF *VIBRIO CHOLERAE* O1 IN BANGLADESHI ADULT PATIENTS WITH CHOLERA

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Immunity against *Vibrio cholerae*, the cause of the severe dehydrating diarrheal illness cholera, is sero-group specific; and sero-grouping is defined by responses to the O-specific polysaccharide (OSP) of the outer membrane lipopolysaccharide (LPS). Despite this, human immune responses to *V. cholerae* OSP have not previously been characterized. To address this, we assessed immune responses against *V. cholerae* OSP in adult patients with cholera caused by *V. cholerae* O1 El Tor Inaba and Ogawa infection in Dhaka, Bangladesh. Inaba patient plasma IgG, IgM, and IgA responses to Inaba OSP and LPS increased significantly from acute to convalescent phase of illness, and correlated well (R=0.86, 0.73, 0.91, p<0.01, respectively). Plasma IgG, IgM, and IgA responses to Ogawa OSP and LPS in Ogawa patients also correlated well (R=0.69, 0.58, 0.92, p<0.01, respectively). Plasma IgM responses to Inaba OSP and Ogawa OSP correlated with respective vibriocidal responses (R=0.80, p<0.001; R= 0.66, p<0.001; respectively). Addition of either OSP or LPS to the vibriocidal assay inhibited the vibriocidal response in a comparable and concentration dependent manner. Mucosal IgA immune responses to OSP and LPS were also similar. Our study is the first to characterize anti-O-specific polysaccharide immune responses in patients with cholera using a purified OSP reagent, and suggests that responses targeting *V. cholerae* LPS, including the vibriocidal responses that correlate with protection against cholera, predominantly target *V. cholerae* OSP. Induction of anti-OSP responses may be associated with protection against cholera, and our results may support the development of a vaccine targeting *V. cholerae* OSP.

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A MASS VACCINATION PROGRAM WITH THE ORAL CHOLERA VACCINE, SHANCHOL, IN DHAKA, BANGLADESH

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A large feasibility and effectiveness study on an oral cholera vaccine, Shanchol™ was conducted in a high risk urban population in Bangladesh to assess the feasibility of delivery and vaccination strategies utilizing the existing national immunization system as well as the effectiveness of the mass campaign. Bangladesh faces biannual peaks of cholera each year with 300,000 severe cases and at least a million infections. With the availability of the affordable and easy to administer Shanchol vaccine, the need to utilize it as a public health intervention in a cholera prone country appeared extremely important in the national and regional context.The feasibility study included vaccination together with a behavior change communication strategy based on a cluster randomized design in urban Mirpur, in six wards with a high cholera hospitalization rate.A geographic information system (GIS) approach was used for the census to identify households and prepare cluster maps using PDAs. A vaccination program was conducted from the 17th of February to the 16th of April, 2011. Consent was obtained and bar coded identification cards were provided to eligible individuals for census updates and to identify them during passive surveillance for cholera. Three arms of the program included a vaccine (n=80,000), a vaccine plus behavior change communication ((n=80,000) as well as a non-intervention control arm(n=80,000).Of the 176,090 eligible population (excluding those aged <1yr and pregnant woman), vaccination was carried out in about 142,000 individuals. About 87% of coverage was obtained for two doses of the vaccine. Thus, over 265,590 doses of Shanchol was delivered in 36 centers in two months. The program was found to be feasible and could be delivered to a high risk densely populated urban area demonstrating that immunization is possible in all age groups using the EPI vehicle of delivery. Hand washing and point of use water treatment interventions are ongoing. Results of the effectiveness of the vaccine and behavior change interventions will be available by the middle of 2013.

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SERO-EPIDEMIOLOGIC SURVEY OF EPIDEMIC CHOLERA IN HAITI TO ASSESS SPECTRUM OF ILLNESS AND RISK FACTORS FOR SEVERE DISEASE

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The variant strain of *Vibrio cholerae* causing the ongoing cholera epidemic in Haiti may be more virulent than previous El Tor strains, which are estimated to cause severe disease in only 2% of those infected. To assess the spectrum of illness from cholera in Haiti and risk factors for severe disease, we conducted a cross-sectional survey in Grande Saline commune, which reported a high cholera attack rate (18%) between October 2010 and April 2011. From March 22-April 6, 2011, we interviewed 2,543 residents ≥2 years old in 1,225 households and collected serum from 2,446 (96%) for blood group, vibriocidal, and anti-cholera toxin (CT) antibody testing. Among participants with watery diarrhea and a positive antibody test, we defined severe cholera by receipt of IV fluids and overnight hospitalization, moderate disease by a health facility (HF) visit without severe disease, and mild disease by no

HF visit. Of 2,543 participants, 541 (21%) reported watery diarrhea since October 2010; 466 (18%) also reported a cholera diagnosis, of whom 157 (34%) had severe cholera. Among 2,446 specimens, 689 (28%) had a positive vibriocidal antibody titer of ≥1:320. Among 1,328 specimens tested to date, 279 (21%) were positive for CT IgG antibodies; 485 (37%) specimens were positive by vibriocidal and/or CT antibody testing. Among these 485 participants, 60 (12%) had severe cholera, 57 (12%) had moderate disease, 32 (7%) had mild disease, and 336 (69%) were asymptomatic. Among antibody-positive participants with watery diarrhea, those with blood group O were more likely to report hospitalization than participants with other blood groups (RR 1.7, 95% CI 1.02-2.8). Exposure to *V. cholerae* was widespread among this population. Blood group O was a borderline risk factor for hospitalization, consistent with findings reported elsewhere. A far greater proportion of participants with evidence of infection had severe cholera (12%) than in previous studies of *V. cholerae* biotype El Tor. Future projections for treatment supply needs (*e.g.*, IV fluids) should account for this elevated severity rate.

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A HISTORICAL LOOK AT THE FIRST REPORTED CASES OF LASSA FEVER: IGG ANTIBODIES FORTY YEARS AFTER ACUTE INFECTION

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Lassa fever is an acute and sometimes severe viral hemorrhagic illness endemic in West Africa. One important question regarding Lassa fever is the duration of IgG antibody after infection. We were able to locate three persons who worked in Nigeria in the 1940-1970s, two of whom were integrally involved in the early outbreaks and investigations of Lass fever in the late 1960s, including the person (Lily Pinneo) from whom Lassa virus was first isolated. Two persons had high titers of Lassa virus-specific IgG antibody over 40 years after infection, indicating the potential for long-term persistence of these antibodies. One person was likely infected in 1952, seventeen years before the first recognized outbreak. We briefly recount the fascinating stories of these three pioneers and their important contribution to our understanding of Lassa fever. A short video interview with Lily Pinneo ("Penny's Story") will be shown (also available at www.vimeo.com/calmdog/pennystory). (Note: Although respect for patient confidentially would normally preclude the use of names, the persons involved in these early outbreaks of Lassa fever were widely publicized in the popular press as well as scientific literature. Furthermore, all three persons or their families provided written permission for the use of their names in this study.)

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RIFT VALLEY FEVER DISEASE RISK MAP FOR KENYA

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We previously used historical data on Rift Valley Fever outbreaks in Kenya to identify 38 of 69 districts located in 6 of 8 provinces, as the regions where the RVF epizootics have occurred in country since 1912. In this study we used semi-quantitative risk assessment to determine likelihood of RVF epizootics for each district in the country. The risk of exposure was evaluated as the proportion of involvement in prior epizootics since 1951 whereas disease outcome was assessed using the prevalence data collected during the 2006/07 outbreak in both humans and animals. Exposure and outcome scores were assigned for each district. To obtain a probability-impact weighted estimate for each district, the exposure and outcome estimates were aggregated. Any district with an aggregate score ≥ 5 (max score = 9) was classified as high risk, districts with score ≥ 2 was classified as medium. Districts that had never reported RVF were low risk. The risk map was then subjected to an expert opinion forum to identify RVF high risk administrative divisions within each district. Bivariate analysis using geographic and geologic variables previously found associated with human cases of RVF in Kenya was used to identify possible risk factors for endemicity. In total 21/69 (30.4%) and 20/69 (29%) districts were classified as high and medium risk, respectively. The remaining 28 districts were classified as being at low risk, including all the districts of Nyanza and Western provinces. Using non-parametric ANOVA, presence of certain soil types (solonetz, calcisol, solonchak and planosols), less than 100mm average annual rainfall during non-El Niño years, altitude below 1160 m, densely bushed areas and presence of agrisparse vegetation were all associated with high risk districts (p-value <0.05) . Overall, 31.1% and 32% of the national livestock (cattle, sheep, goats, camels) were in high and medium risk districts, respectively. For stepwise livestock vaccination during predicted epidemics, the country would require less than US$1 million to buy vaccines for all livestock in high risk districts. This risk assessment map provides a scientific basis for developing stepwise vaccination program against RVF during years of epidemic threat. Identification of possible risk factors associated with RVF endemicity could assist other countries at risk within the RVF prone regions to develop country-specific risk maps for use in prioritizing limited resources.

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INNATE IMMUNE GENE POLYMORPHISMS ARE ASSOCIATED WITH HUMAN RIFT VALLEY FEVER DISEASE

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In recent years there have been multiple outbreaks of Rift Valley Fever (RVF) in Kenya resulting in significant human morbidity and mortality. Goals of this study were to identify probable symptom groups indicative of RVF and to elucidate potential genotypic frequencies associated with RVF clinical disease. We conducted a cross-sectional cluster survey among residents (N=1,080; 1-85 yrs) in 6 villages in Northeastern Province, Kenya. Participants underwent questionnaire administration, physical exam, vision testing, and blood collection. Single nucleotide polymorphism (SNP) genotyping was performed on two subsets: 200 unrelated subjects and 200 subjects who reported clinical symptoms consistent with past RVF. Four symptom clusters were defined: meningoencephalitis, hemorrhagic fever, eye disease and RVF-not otherwise specified. SNPs in 48 viral sensing and response genes were investigated. Association analysis was conducted between SNP genotype and RVF symptom clusters as well as positive RVF serology. Positive serology was significantly associated with: DHX58/LGP2: rs2074158 (p=0.08) and rs2274863 (p=0.03), and TLR8: rs57474080 (p=0.08). The meningoencephalitis phenotype among positive patients was associated with: DDX58/RIG-I: rs2274863 (p=0.02) and TLR8: rs5744081 (p=0.01). Associations between frequency and having any RVF symptoms were significant for: TLR8: rs3747414 (p=0.05) and rs5744081 (p=0.06); and TLR7: rs864058 (p=0.07). Having three or more symptoms was significant with: TICAM1/TRIF: rs2292151 (p=0.09); MAVS: rs17857295 (p=0.03); IFNAR1: rs17875834 (p=0.02) and rs17875863 (p=0.00); and DDX58/RIG-I: rs1133071 (p=0.08). SNPs significantly associated with eye disease were: TLR8: rs5744077 (p=0.05), rs5744084 (p=0.05) and rs5744088 (p=0.02). Of the 48 SNPs tested, TLR7, TLR8, and RIG-I were repeatedly associated with the RVF symptom

groups, suggesting that these genes may have a robust association with RVFV-associated outcomes. Future analyses will include allelic association, analysis of haplotypes, and inclusion of advanced ophthalmologic results.

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INFECTION AND TRANSMISSION OF RIFT VALLEY FEVER VIRUSES LACKING THE NSS AND/OR NSM GENES IN MOSQUITOES: POTENTIAL ROLE FOR NSM IN MOSQUITO INFECTION

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Rift Valley fever virus is an arthropod-borne human and animal pathogen responsible for large outbreaks of acute and febrile illness throughout Africa and the Arabian peninsula. Reverse genetics technology has been used to develop deletion mutants of the virus that lack the NSs and/or NSm virulence genes and have been shown to be stable, immunogenic and protective against Rift Valley fever virus infection in animals. We assessed the potential for these deletion mutant viruses to infect and be transmitted by *Aedes* mosquitoes, which are the principal vectors for maintenance of the virus in nature and emergence of virus initiating disease outbreaks, and by *Culex* mosquitoes which are important amplification vectors.*Aedes aegypti* and *Culex quinquefasciatus* were fed bloodmeals containing the deletion mutant viruses. Two weeks post-exposure mosquitoes were assayed for infection, dissemination, and transmission. In *Ae. aegypti*, infection and transmission rates of the NSs deletion virus were similar to wild type virus while dissemination rates were significantly reduced. Infection and dissemination rates for the NSm deletion virus were lower compared to wild type. Virus lacking both NSs and NSm failed to infect *Ae. aegypti*. In *Cx. quinquefasciatus*, infection rates for viruses lacking NSm or both NSs and NSm were lower than for wild type virus. In both species, deletion of NSm or both NSs and NSm reduced the infection and transmission potential of the virus. Deletion of both NSs and NSm resulted in the highest level of attenuation of virus replication. Deletion of NSm alone was sufficient to nearly abolish infection in *Ae. aegypti* mosquitoes, indicating an important role for this protein. Barriers to infection and dissemination of the NSm deletion mutant were further investigated by comparing sagittal sections of *Ae. aegypti* infected with wild type or the NSm deletion virus. The double deleted viruses represent an ideal vaccine profile in terms of environmental containment due to lack of ability to efficiently infect and be transmitted by mosquitoes.

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ECONOMIC ANALYSIS OF ALTERNATE RIFT VALLEY FEVER CONTROL OPTIONS FROM A MULTISECTORAL PERSPECTIVE

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Rift Valley fever is a viral zoonosis that primarily affects people, cattle, sheep, goats, camels, buffalos, dromedaries, antelopes and wildebeest. The two most recent RVF epidemics in Kenya occurred in 1997/1998 and in 2006/2007 with severe socio-economic consequences in multiple sectors of the national economy. This study was undertaken to provide policy evidence on cost-effectiveness and benefits associated with alternate control options as well as the one health institutional arrangements for its improved prevention and control from both a public health and livestock perspectives. The approach employed multistage process that involved; mapping of one health stakeholders; an institutional analysis; simulation of different options (combinations of vaccination, sanitary measures, surveillance, vector control and awareness campaigns) using an individual-based epidemiological model and economic modeling to estimate costs of control per disability adjusted live year averted and benefits to the livestock sector and national economy. Up to 28 different agencies are relevant and need to be considered in one health collaborations to RVF prevention and control. The stakeholders go beyond the line animal and public health sectors. Socio network analysis reveals denser networks and stronger relational linkages between the public health stakeholders while the reverse is true for animal health stakeholders. Centrality statistics measures of Degree, Betweeness and Closeness identified the two health sectors, and the community as being the actors who linked clusters within the network. A non health ministry emerged as the actor demonstrating the highest closeness. The study concludes that a narrow scope of one health approach through collaboration of animal and human health agencies leaving out other non health actors and the livestock keepers could weaken control of zoonoses. Preliminary cost-benefit analysis of animal vaccination demonstrates good returns to investment (cost benefit ratio of greater than 1).

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MOUYASSUÉ VIRUS, A HIGHLY DIVERGENT HANTAVIRUS IN THE BANANA PIPISTRELLE (*NEOROMICIA NANUS*) IN CÔTE D'IVOIRE

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Newfound hantaviruses detected in multiple species of shrews and moles (Order Soricomorpha) across four continents are genetically more diverse than those harbored by rodents (Order Rodentia), suggesting that the host range of hantaviruses may be more expansive than previously imagined. In particular, mammals having shared ancestry with soricomorphs, such as bats (Order Chiroptera), may have figured prominently in the diversification of hantaviruses by virtue of their rich biodiversity, vast geographical range, and demonstrated ability to host myriad viruses. To investigate this possibility, either frozen, ethanol-fixed or RNAlater®-preserved tissues from 323 bats (representing 21 genera and 32 species), captured in Asia, Africa and the Americas in 1981-2012, were analyzed for hantavirus RNA by RT-PCR. After numerous failed attempts, hantavirus RNA was detected in ethanol-fixed liver tissues from two of 12 banana pipistrelles (*Neoromicia nanus*), captured during June 2011 near Mouyassué village, in Côte d'Ivoire. Analysis of a 1,691-nucleotide region of the RNA-dependent RNA polymerase-encoding L segment of the newfound hantavirus, designated Mouyassué virus (MOUV), exhibited nucleotide and amino acid sequence similarity of less than 71% to all representative soricomorph- and rodent-associated hantaviruses. MOUV sequences were identical in two banana pipistrelles, a male-female pair captured simultaneously and presumed to be a mating couple, suggesting horizontal virus transmission or common-source infection. Phylogenetic analysis, using maximum likelihood and Bayesian methods, showed that MOUV formed a highly divergent lineage, distant from all other hantaviruses, except Magboi virus recently detected in the hairy slit-faced bat (*Nycteris hispida*) from Sierra Leone. Suboptimal primer design and imperfect cycling conditions may have been responsible for the failure to detect hantavirus RNA in other insectivorous bat species. Also, efforts to obtain the full genome of MOUV may have been hampered by poor RNA preservation in ethanol-fixed tissues. Nevertheless, the detection of hantavirus RNA in ethanol-fixed tissues should make available a larger

pool of archival tissues for future exploratory studies of hantaviruses in bats and other insectivorous small mammals, such as hedgehogs (Order Erinaceomorpha).

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DIFFERENTIAL IMMUNE RESPONSES IN DEER MICE (*PEROMYSCUS MANICULATUS*) EXPERIMENTALLY INFECTED WITH SIN NOMBRE OR ANDES VIRUSES

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Deer mice (*Peromyscus maniculatus*) are the principal reservoirs of Sin Nombre virus (SNV), which causes the great majority of hantavirus cardiopulmonary syndrome (HCPS) cases in North America. Infection of rodent reservoirs results in life-long persistence without signs of disease or tissue pathology. Andes virus (ANDV), which causes the great majority of HCPS cases in South America, is naturally hosted by the long-tailed pygmy rice rat (*Oligoryzomys longicaudatus*) and similarly causes persistent infection without disease in its host. We developed a bioinformatics approach for designing real-time PCR arrays using the unannotated deer mouse genome to examine the expression of immune genes of deer mice infected with either SNV or ANDV. While deer mice remained persistently infected with SNV without disease, deer mice infected with ANDV cleared the virus but also without disease. We determined that many of the same genes were expressed in either infection but that levels of expression were substantially higher in ANDV-infected deer mice. These results suggest a quantitative effect in host response to a reservoir hantavirus (SNV) compared to a nonreservoir hantavirus (ANDV) and is associated with persistence or clearance.

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CIMEX LECTULARIUS (BED BUG) MORBIDITY AND MORTALITY AFTER EXPOSURE TO THE DRUG IVERMECTIN

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We show that *Cimex lectularius* (bed bug) suffer high morbidity and mortality when fed on blood containing ivermectin *in vitro*, *in vivo*, and in four human study subjects. Mortality for adult bed bugs was 0% when fed on 0 ng/mL ivermectin (control; n=45) and 100% (n=44) for 260ng/mL ivermectin at day 13 using an artificial feeding membrane. Mortality for *C. lectularius* 3rd and 4th stage instar nymphs that fed on heparinized mouse blood containing 0ng/mL ivermectin (control; n=45) was 0% compared to 95% for 260ng/mL (n=37) at day 13. Combined mortality for adult and nymph bed bugs that fed on mice injected intraperitoneally with the human equivalent dose of ivermectin 0mcg/kg (control) (n=21) was 0% compared to 86% (n=22) in the 200mcg/kg ivermectin group. None of the surviving nymphs exposed to ivermectin molted by day 75 compared to 80% of nymphs in the control group who molted by day 8. Bed bugs that fed once on human study subjects three hours after they had consumed 200mgc/kg of oral ivermectin had a 50% two-day and a 63% (n=24) 20-day mortality rate compared to 4% and 8% (n=24), respectively in the control group. By day 20, 67% (8/12) of the control-group nymphs had molted compared to 0% (0/12) of the bed bugs which fed on human subjects three hours after they had consumed ivermectin. A single exposure to ivermectin can cause bed bug morbidity and mortality. It can also prevent nymph molting. It is possible that ivermectin could be used to help eradicate a bed bug infestation.

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ATOPY, ASTHMA AND SCABIES

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In many remote Australian Indigenous communities' high levels of atopy exist and importantly asthma is now reported as the second most commonly experienced chronic health condition in Indigenous Australians. In many of these same remote communities scabies is endemic. Numerous studies show cross antigenicity exists between house dust mites (HDM) and scabies mites; however to what extent cross allergy contributes to the high prevalence of atopy reported in these communities is unknown. Skin prick tests for atopy use ill-defined allergen extracts whereas component resolved diagnostics (CRD) employs panels of purified allergens to identify major and minor allergens in people with asthma. Such studies have revealed people in Australia allergic to Dermatophagoides pteronyssinus typically produce high titres of IgE antibody to the major allergens Der p 1 and Der p 2. A recent study using CRD to examine the allergenic profile of IgE binding in sera from HDM atopic individuals living in a remote Western Australian Indigenous community revealed high level IgE antibody binding was not directed at the expected Der p 1 and Der p 2 allergens but primarily with Der p 4 (amylase). Significantly when HDM CRD was undertaken on sera collected from scabies infected Indigenous people a markedly similar IgE antibody binding profile was observed, with the majority of cross reactive IgE binding to Der p 4. Individuals with past exposure to scabies but no current infection had the same binding pattern reported for Indigenous HDM atopic individuals, with IgE primarily binding to the Der p 4. The presence of cross reactive antibody to Der p 4 in people with scabies exposure strongly suggests the Group 4 allergen, amylase, is a major cross reactive protein of scabies mites and HDMs and could play a cross sensitisation or cross protective role in the development of atopy and asthma.

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SKIN IMMUNE RESPONSES TO *SARCOPTES SCABIEI:* A ROLE FOR IL-17 IN PATHOGENESIS OF CRUSTED SCABIES?

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Crusted scabies is a severe clinical manifestation of *Sarcoptes scabiei* infection, resulting from a failure of the immune system to control mite proliferation. Detailed understanding of scabies immunopathology, particularly in crusted scabies, has been precluded by the inability to undertake longitudinal studies of infection in humans. Pigs are an excellent animal model for scabies as they are a natural host of *S. scabiei*, and show similar clinical, epidermal, and immunologic changes to humans. Moreover, crusted scabies can be readily established in pigs by treatment with the glucocorticoid dexamethasone (DEX). We undertook a prospective study with 24 pigs in four treatment groups: a) Scabies+/DEX+, b) Scabies+/DEX-, c) Scabies-/DEX+ and d) Scabies-/DEX-. Pigs were scored for lesion development and parasite burden, and skin biopsies collected at monthly intervals. Histological profiling and qRT-PCR was undertaken to compare cellular changes and transcription of key Th1, Th2, and Th17 cytokines. A range of clinical responses to *S. scabiei* were observed in both DEX treated and non-immunosuppressed pigs. An

association was confirmed between disease severity and transcription of the Th2 cytokines IL-4 and IL-13, which were significantly increased 1-3 months post infection. We also observed significant up-regulation of the Th17 cytokines IL-17 and IL-23 in pigs with crusted scabies. Immunohistochemistry showed high numbers of lymphocytes and mast cells, and strong staining for IL-17. While an allergic Th2 type response has been previously described, this is the first evidence suggesting that the Th-17 pathway may also contribute to disease pathogenesis in crusted scabies. This work provides further insights into the characteristics of a dysregulated immune response in crusted scabies, and may lead to new treatment strategies to protect vulnerable subjects from contracting recurrent crusted scabies.

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THE EFFECTS OF CLIMATE ON HUMAN PLAGUE INCIDENCE IN MADAGASCAR

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Connections between climate and vector-borne diseases are well established and various global climate phenomena such as the El Niño Southern Oscillation (ENSO) have been identified to be a strong influence on the epidemiology of various infectious diseases. Vector-borne diseases are often associated with certain time periods and geographical areas with wide-ranging implications for public health. Plague is a vector-borne zoonosis and in Madagascar human plague still has a high prevalence and is undeniably its most important rodent-linked disease. The seasonality in human cases suggests a link to climate and understanding the processes involved is important to enable better disease prediction and effective prevention. The climate in Madagascar is heavily shaped by the ENSO and the Indian Ocean Dipole (IOD). In this study the association between these two large climate drivers and human plague incidence between 1956 and 2008 in Madagascar was investigated. Wavelet analysis was chosen as a qualitative method to identify periods in the last 50 years where links between climate and plague incidence can be suggested. The results show a changing relationship between human plague incidence and climate most likely mediated by changes in the strength and timing of ENSO and the IOD in the 1990s. The correlation between ENSO and plague turned from negative to positive and the association with the IOD became stronger with time. Any of these established associations between large scale climate events and plague incidence are most likely due to the influence of ENSO and the IOD on temperature and precipitation. These are known to affect host and vector ecology as well as transmission potential. The findings not only demonstrate the importance of climate for plague epidemiology but provide the means to explain and predict plague occurrence in Madagascar. This is essential for a country with limited resources to fight infectious diseases.

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ROLE OF TRANSOVARIAL TRANSMISSION IN PERPETUATING THE DEER TICK MICROBIAL GUILD

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In the northeastern U.S., deer ticks maintain a diverse guild of potentially zoonotic microbes, including at least 2 *Borrelia* spp., 2 *Babesia* spp., *Anaplasma phagocytophilum, Rickettsia* sp. and a flavivirus. The mode of perpetuation for most of these agents usually includes horizontal transmission to a mammalian host. Although Borrelia miyamotoi is known to be vertically transmitted (inherited) and *B. burgdoferi* sensu stricto is not, the role of transovarial transmission of other members of the deer tick microbial guild remains poorly explored. Accordingly, we determined the transovarial transmission rate (TOTR, proportion of infected females giving rise to at least one infected individual progeny) for the deer tick guild. Engorged female deer ticks were removed from hunter killed deer from southern Massachusetts and allowed to oviposit. The spent females and samples of resulting larvae were analyzed by PCR or RT-PCR for evidence of infection using group specific primer sets with confirmation of identity by the use of species specific primers. Of the 60 pairs (females and larvae) examined to date, only Babesia odocoilei and a Rickettsia symbiont (ISS) (prevalence 12% and 100%, respectively) appeared to be vertically maintained, with TOTR of 71% and 98% respectively. We conclude that transovarial transmission is not a frequent mode of perpetuation for most members of the deer tick microbial guild, a conclusion that is consistent with the epidemiological evidence for a general absence of acute human infection during the months of peak larval deer tick activity.

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EMERGENCE PATTERNS OF *BABESIA MICROTI* IN *IXODES SCAPULARIS* TICKS AND HUMANS IN NEW ENGLAND

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Human babesiosis, an emerging tick-borne disease primarily caused by the intraerythrocytic protozoan *Babesia microti*, is expanding geographically in the northeastern United States. To determine the relationship between *B. microti* infection in *Ixodes scapularis* ticks and humans over the expanding range of human babesiosis, we compared tick and human infection rates of *B. microti, B. burgdorferi*, and both together in an emerging *B. microti* area in northern Connecticut and long-established, highly endemic areas for human babesiosis in southern Connecticut and Nantucket, Massachusetts. We found that B. burgdorferi was more prevalent in ticks than *B. microti* in all sites except southern Connecticut where they were similarly represented. Tick infection prevalence with B. microti at the northern edge of reported human babesiosis distribution was lower than at the other sites in 2010, but reached similar prevalence in 2011, suggesting rapid establishment of the pathogen in this emerging area. By comparing the *B. burgdorferi:B. microti* infection ratio in ticks with the Lyme disease:babesiosis incidence rate ratio in humans, we estimated that exposure to *B. burgdorferi*-infected ticks is 2-2.6 times more likely to result in a diagnosed/reported human case than exposure to *B. microti*-infected ticks in endemic areas and 6.5 times more likely in emerging areas. These data suggest that there may be geographic variation in *B. microti* infectivity to humans and that human babesiosis is markedly underdiagnosed and/or underreported in emerging areas. Entomological surveillance can provide an early warning system for human risk for babesiosis in such areas.

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BORRELIA BURGDORFERI COINFECTION ENHANCES *BABESIA MICROTI* INFECTION IN WHITE-FOOTED MICE AND TRANSMISSION TO *IXODES SCAPULARIS* TICKS

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Babesia microti and *Borrelia burgdorferi,* the primary agents of human babesiosis and Lyme disease, are both transmitted by *Ixodes scapularis*

ticks to white-footed mice (*Peromyscus leucopus*) and to humans. To determine whether co-infection increases intensity of infection in *P. leucopus* or transmission to feeding ticks, we infested *Peromyscus leucopus* mice with nymphal *I. scapularis* ticks infected with either *B. microti* or *B. burgdorferi* alone, or coinfected with both simultaneously or sequentially. We then assessed intensity of infection in mice and the transmission of *B. microti* and *B. burgdorferi* infection to ticks at 1, 2, 3, 4, and 6 weeks after mouse infection. Mice were infested with infected nymphal ticks and intensity of infection measured by *B. microti* parasitemia in blood and *B. burgdorferi* DNA in bladder, heart, and joint tissue. Transmission of infection to ticks was assessed by placing uninfected larval ticks on the same infected mice and assaying for both pathogens in ticks after they fed and molted to the nymphal stage. We found that *B. microti* parasitemia was greater in *B. microti/B. burgdorferi*-coinfected mice than mice infected with *B. microti* alone. The percentage of ticks that became infected with *B. microti* was greater in those fed on coinfected mice compared to those fed on mice infected with *B. microti* alone. In contrast, coinfection did not uniformly increase *B. burgdorferi* tissue burden or transmission to ticks. We conclude that *B. microti/B. burgdorferi* coinfection increases *B. microti* parasitemia and transmission to larval ticks in the natural mouse reservoir host. These findings suggest that the presence of *B. burgdorferi* infection may increase the incidence of human babesiosis in endemic areas and may be a prerequisite for *B. microti* enzootic or endemic establishment in selected regions.

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IS HAVING TOO MUCH JNK ALL THAT BAD: POTENTIAL ROLE FOR MODULATION OF INSULIN SIGNALING IN VECTOR COMPETENCE

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Two populations of *Aedes aegypti*, Moyo-R and Moyo-S, show differences in susceptibility to dengue virus (DENV) with Moyo-S being significantly larger, as determined by wing length measurements, and more susceptible to infection than Moyo-R. Regulation of insulin/insulin-like signaling (IIS) during larval development determines fitness, including body size, of the adult mosquito. During favorable environmental conditions, c-Jun N-terminal Kinase (JNK) is inactive, regulated by Puckered (Puc) phosphatase. Puc dephosphorylates key Tyrosine and Threonine residues on its Thr-Pro-Tyr motif. However, in unfavorable conditions Puc activity is diminished allowing JNK signal transduction pathway to initiate slower organismal growth by controlling IIS. Our hypothesis is that RNAi knock down of *puc* in the Moyo-S population will result in increased JNK activity resulting in smaller mosquitoes, due to modulating components of IIS, with an increase in pathogen defense. RNA was extracted from 2nd, 3rd, 4th instar, pupal, and adult stages, *puc* and *jnk* gene expression were validated via qRT-PCR, and wing lengths of adult females at 3 days post-emergence were measured. Data were compared between Moyo-S and Moyo-R populations. Endogenous levels of *puc* at 2nd and 3rd instars and adult stages in the Moyo-S population were significantly higher, $p < 0.05$, compared to that of the Moyo-R population. Endogenous levels of *jnk* were significantly lower, $p < 0.05$, in the Moyo-S population compared to expression at all stages of development in the Moyo-R population. The smaller sized Moyo-R population expresses greater endogenous levels of *jnk* and lower endogenous levels of *puc*. This is what we would expect in an organism whose normal gene expression of *jnk* mimics an active stress response. It is possible the expression of *puc* and *jnk* seen in Moyo-R is key to its refractoriness to pathogens. However, there may also be associated pathway(s) linked to *jnk* signal transduction pathway important to the observed difference in vector competence between the populations.

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SAND FLY SPECIES COMPOSITION IN A RURAL SETTING WITH HYPERENDEMIC CUTANEOUS LEISHMANIASIS TRANSMISSION

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American Cutaneous Leishmaniasis, ACL, is a zoonotic disease with a large richness of co-occurring vector species in transmission settings. Here, we describe the patterns of phlebotomine sand fly (Diptera: Psychodidade) species diversity at Comunidad de Trinidad Las Minas, Capira, Panamá, an hyperendemic focus of ACL transmission. Our study setting consisted of 24 houses, 12 subjected to two rounds of insecticide thermal fogging with and the other 12 kept as control. During 15 months (April 2010 - June 2011) we monitored Sand Fly species composition and abundance with CDC light traps inside the houses (domicile) and outside (peridomicile). We collected a total of 5628 Sand Flies, and we were able to identify 5617 of the samples into 24 species, a number of species close to 25 ± 1.6, the estimate from the Chao2 Index. The most abundant species were *Lutzomyia gomezi* (20%), *L. triramula* (20%) and *L. trapidoi* (20%). Cluster analyses showed that most of the 24 houses had high similarity in abundance patterns of the six most common sand fly species, with only few peripheral houses not following the main cluster pattern. We also found that species richness was decreased to 22 species in the fogged houses, of which only 19 were found in the domiciliary environment. Changes in species richness were especially notorious at the end of the wet season. Our results suggest that species richness can be decreased following insecticide thermal fogging in the domiciliary environments, primarily affecting the less common species.

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SECRETED SCABIES MITE COMPLEMENT INHIBITORS PROMOTE STAPHYLOCOCCAL EVASION FROM PHAGOCYTOSIS

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Staphylococcus aureus is a versatile and harmful pathogen causing infections ranging from superficial to systematic infections. The ability of *S. aureus* to quickly develop and maintain resistance to clinically available antibiotics has resulted in the global epidemic of multi-drug resistant strains. In the Australian Aboriginal population, extreme incidences of *S. aureus* infections are associated with high prevalence of superficial skin infections caused by the scabies mite, *Sarcoptes scabiei*. Scabies mites cause mechanical infringement by burrowing into the upper epidermis and colonisation of *S. aureus* in these skin burrows has been reported. The human complement system is an immediate defence against the invading pathogen. As a successful pathogen, *S. aureus* produces an array of complement evading molecules. Interestingly, scabies mites also produce several families of different protein classes that interfere with various host complement molecules. They are secreted into the mite gut and excreted into the epidermal burrows with the faeces. We postulated that scabies mite complement inhibitors create a microenvironment that promotes bacterial survival. Here we investigate the effect of recombinant scabies mite complement inhibitors on staphylococcal *in vitro* survival. Firstly, we addressed this in whole blood bactericidal assays and showed that two mite complement inhibitors increased the survival of *S. aureus* in a concentration dependent manner. We have additional data indicating that these complement inhibitors reduce the opsonisation of *S. aureus*. We are currently assessing the effect of the mite molecules on phagocytosis of *S. aureus* by FACS. Subsequently, we aim to develop an *in vivo* study in our porcine model on the influence of scabies mites complement

inhibitors on *S. aureus* survival. We postulate that comprehending the interactions between mite complement inhibitors and bacteria will foster the development of novel interventions.

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FIELD USER ACCEPTABILITY EVALUATION OF A NOVEL, SELF-SUPPORTING, LONG-LASTING INSECTICIDAL NET

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Insect bed nets provide protection against arthropod-borne disease pathogens such as malaria, dengue, and leishmaniasis. United States Army service members currently have a choice between two types of bed nets to use in field environments; however, both have various limitations that preclude effective long-term use by non-mobile forces. Therefore, the US Army was faced with a challenge to develop an improved bed net that does not have any of the limitations associated with these existing bed nets. The Walter Reed Army Institute of Research has partnered with Tritons Systems, Inc. to develop a novel, self-supporting, long lasting, insecticide-impregnated net (LLIN). The purpose of this study was to evaluate the new bed net in comparison with the existing Standard and Self-Supporting Low-Profile bed nets using an acceptability threshold of 70%. Upon completion of a large scale field training exercise in which these bed nets were used over the course of several nights, soldiers completed a self-administered survey answering questions about their ease of use, setup, dismantling, and comfort. Results of this acceptability study will be presented in the context of US Army force health protection.

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APOPTOSIS OF *ASCOGREGARINA TAIWANENSIS* (APICOMPLEXA: LECUDINIDAE) WHICH FAILED TO MIGRATE WITHIN ITS NATURAL HOST

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Sexual reproduction of *Ascogregarina taiwanensis* (Apicomplexa: Lecudinidae), a parasite specific to the mosquito *Aedes albopictus*, in malpighian tubules is initiated by the entry of trophozoites developed in the midgut shortly after pupation (usually < 5 h). However, only a low proportion of trophozoites are able to migrate; others end up dying. In this study, we demonstrated those trophozoites which failed to migrate eventually died of apoptosis. Morphological changes such as shrinkage, chromatin aggregations, and formation of blunt ridges on the surface were seen in moribund trophozoites. In addition, DNA fragmentation of trophozoites isolated from the midgut of pupae was demonstrated by the presence of DNA ladders, Annexin V staining, and TUNEL assays. Due to detection of caspase-like activity, apoptosis of those trophozoites may have occurred through a mechanism of an intrinsic or mitochondrial-mediated pathway. Although apoptosis was observed in various species of protozoa, it provides a challenge to evolution as cell death might not be beneficial for the perpetuation of a species. However, it is believed that apoptosis may regulate the parasite load of *A. taiwanensis* within its natural mosquito host, leading to an optimized state of the survival rate for both parasite and host.

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DEVELOPMENT OF AN INTEGRATED PUSH-PULL SYSTEM FOR VECTOR CONTROL

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Push-pull vector control strategies use a repellent compound to "push" arthropods away from hosts and an attractant to "pull" arthropods into traps. The objective of this study is to develop an integrated push-pull vector control system that is effective against mosquitoes, sand flies, and other arthropod vectors of disease. To this end, we have evaluated a number of commercial off the shelf repellent and attractant compounds against *Anopheles stephensi*, *Aedes aegypti*, *Phlebotomus papatasi*, and *Lutzomyia longipalpis*. The first year of the study focused on laboratory work in which repellent and attractant compounds were first tested for spatial repellency or attractiveness in a modified choice chamber system, as reported previously. The most successful compounds from the choice chamber trials were then selected for wind tunnel assays. In the second and third years of the study, repellent and attractant compounds selected from the choice chamber and wind tunnel trials will be used in field trials in Africa, South America, and Asia. This presentation will focus on the results of the study to date.

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COMPLEMENT INHIBITORS FROM SCABIES MITES PROMOTE STREPTOCOCCAL SURVIVAL

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In tropical settings infection with *Sarcoptes scabiei* mites predisposes to secondary bacterial skin infections, caused by *Streptococcus pyogenes* (GAS). In Australia scabies is highly prevalent in Aboriginal communities, and rheumatic fever and rheumatic heart disease prevalence has reached 2%, translating to the highest incidences reported globally. Scabies and pyoderma have also been linked with outbreaks of acute post-streptococcal glomerulonephritis. In some remote Aboriginal communities up to 70% of children presented with scabies and skin sores by one year of age. Community-wide treatment of scabies decreases pyoderma, pointing towards a key role of the mite burrowing in the human epidermis. Our study aims to reveal the molecular mechanisms underlying the link between scabies and associated bacteria.To evade the immediate host immune response multiple scabies mite intestinal proteins disrupt the complement cascade at several levels, primarily to prevent complement-mediated damage of the mite gut epithelium. We described two distinct families of scabies mite intestinal proteins that interfere with the human complement system, consisting of catalytically inactive serine proteases and serine protease inhibitors. We hypothesized that upon excretion into the epidermis the increased level of anti-complement activity has an effect on the bacteria that colonize the burrows. We showed the effect of scabies mite complement inhibitors on human complement in hemolytic assays, ELISA-based complement activation assays and complement binding assays. We demonstrated in human whole blood assays that each of four scabies mite complement inhibitors tested increased GAS survival rates considerably in a dose dependent manner. This is the first molecular study suggesting a mechanism that may contribute to the positive association between scabies and GAS skin infection, thereby emphasizing the potential worth of a concerted intervention against scabies in the control of secondary bacterial skin infections.

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EXPERIMENTAL ACQUISITION, DEVELOPMENT, AND TRANSMISSION OF *LEISHMANIA TROPICA* BY *PHLEBOTOMUS DUBOSCQI*

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We report experimental infection and transmission of *Leishmania tropica* (Wright), by the blood-feeding sand fly *Phlebotomus duboscqi* (Neveu-Lemaire). Groups of laboratory-reared female sand flies that fed "naturally" on *L. tropica*-infected hamsters, or artificially via membrane feeding device, on a suspension of *L. tropica* amastigotes, were dissected at progressive time points post-feeding. Acquisition, retention

and development of *L. tropica* through procyclic, nectomonad, and leptomonad stages to the infective metacyclic promastigote stage, and anterior progression of the parasites from abdominal midgut blood meal to the thoracic midgut were demonstrated in both groups. Membrane feeding on the concentrated amastigote suspension led to metacyclic promastigote infections in 60% (56 of 93) of sand flies, whereas only 3% (4 of 118) of *P. duboscqi* that fed naturally on an infected hamster developed metacyclics. Sand flies from both groups re-fed on naïve hamsters, but despite infections in 25-50% of membrane-fed and 2-3.5% of naturally-fed flies, no skin lesions developed in the hamsters. After four months of observation these animals were euthanized and necropsied. Screening of the organs and tissue by polymerase chain reaction (PCR) that targeted the small subunit RNA gene, amplified generic *Leishmania* DNA from liver, spleen, bone marrow, and blood, but only from hamsters bitten by membrane-infected *P. duboscqi*. These results are notable in demonstrating the ability of *P. duboscqi,* originating from Kenya, to acquire, retain, develop, and transmit a Turkish strain of *L. tropica* originally isolated from a human case of cutaneous leishmaniasis. This marks the first demonstration of complete development and transmission of *L. tropica* by a member of the *Phlebotomus* subgenus of sand flies.

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DEVELOPING ONTOLOGIES FOR VECTORS AND VECTOR-BORNE DISEASES

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In the frame of the NIAID-funded VectorBase project we have been developing ontologies for vector-borne diseases as well as for other domains that are related to that one (e.g. vectors, insecticide resistance, etc.). These ontologies, originally developed using the OBO format, but now moving to the widely used OWL format, can be used for a variety of purposes, most importantly for the development of intelligent IT tools that can help control this kind of diseases. For example, ontologies can be used in order to enhance the search possibilities of specific databases, especially since, when using specific schemata (e.g. Chado), they can also drive the databases as such. Staying in the area of databases, ontologies also help tremendously when it comes to achieving interoperability between different databases that share a part of the data (and/or the metadata). Best example for the this would be two databases (e.g. VectorBase and EuPathDB) that have vector-borne diseases as a common theme, but which are centered around a different primary object (here, vector and pathogen). Finally, ontologies are helpful in the design and implementation of epidemiological IT tools (e.g. decision-support systems), since they can be used both to model specific research cases and to ascertain consistency in terminology, also taking care of multiple synonyms. In our case we are presently focusing on the construction of ontologies for Dengue fever and Chagas' disease. Both of them are modeled after the malaria ontology (IDOMAL) previously developed, which was constructed as an extension to the Infectious Disease Ontology IDO. We hope that the availability of these (and future) ontologies will help develop novel, efficient epidemiological tools for the fight against vector-borne diseases.

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"TSETSE TRAPS AND WHY DO WE FEAR THEM?" COMMUNITY-CENTRED TSETSE CONTROL IN UGANDA

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There is renewed vigour in efforts to eliminate neglected tropical diseases including sleeping sickness (human African trypanosomiasis). Towards this end, efforts are being made to develop more cost-effective methods of tsetse control. In the West Nile region of Uganda, novel designs of insecticide-treated target are being deployed over an area of ~250 km^2. The operational area covers villages where tsetse control has not been conducted previously. The effectiveness of the targets will depend, in part, on their acceptance by the local community. Accordingly, we assessed knowledge, perceptions and acceptance towards targets in villages where targets and traps had or had not been used previously. Sixteen Focus group discussions with male and female participants were conducted in eight villages across Arua District. Discussions were audio recorded, transcribed and translated. We used thematic analysis to compare the views of both groups and identify salient themes. Preliminary findings indicate that despite the villages being <10 km apart, community members perceived deployed baits very differently. Villagers who had never seen traps before expressed fear, anxiety and panic when they first encountered them. This was related to associations with witchcraft and "ghosts from the river" which are traditionally linked with physical or mental illness, death and misfortune. By contrast, villagers living in areas where traps had been used previously had positive attitudes towards them and were fully aware of their purpose and benefits. The latter group reported that they had similar negative perceptions when tsetse control interventions first started a decade ago. Our results suggest that despite their apparent proximity, acceptance of traps varies markedly between villages and this is related to the duration of experience with tsetse control programs. The success of community-based interventions against tsetse will therefore depend on early sensitisation campaigns that reach all communities, especially those living in the areas new to such interventions.

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CLIMATIC CHANGES IN THE PREVALENCE OF *LEISHMANIA DONOVANI* INFECTION WITHIN THE NATURAL POPULATION OF SAND FLY VECTOR SPECIES INFLUENCES THE TRANSMISSION PATTERN OF VISCERAL LEISHMANIASIS

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Visceral Leishmaniasis is one of the major life threatening infectious diseases in the Indian subcontinent, transmitted by the bite of female sand flies. Measuring the infectivity in the vector population collected in different seasons may be useful for understanding the transmission dynamics of a vector borne disease as well as suitable season for applications of the vector elimination programmes. Sand flies were collected from the endemic regions of Bihar state, India in three consecutive seasons. *Leishmania donovani* infection was detected in 1397 female *Phlebotomus argentipes* using PCR targeting *Leishmania* specific minicircle of kDNA region. Further parasitic load in the infected sand flies were measured using quantitative PCR. Sand flies were found to be maximum infected in the season of winter followed by rainy and summer that affects the VL transmission.

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INTEGRATED ENTOMOLOGICAL SURVEILLANCE IN ZAMBIA: INTRODUCTION OF A PHASED PROGRAM FOR DISTRICT BASED DELIVERY THROUGH ENVIRONMENTAL HEALTH TECHNOLOGISTS

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Zambia has witnessed a rapid expansion in the delivery of insecticidal based interventions such as Indoor Residual Spraying (IRS) and Long Lasting Insecticidal Nets (LLINs). Despite the intensification of vector control programming, entomological surveillance is conducted sporadically and is geographically limited in its coverage. Currently, there is no routine longitudinal surveillance system that monitors the entomological impact of vector control interventions. A conceptual framework based on

phased delivery of individual components of an integrated entomological surveillance system has been designed, along with tools to support district-level ongoing vector control activities. Individual components of the overall program support training of new and existing recruits, data management, both intra- and inter-district program performance, species composition mapping, and vector bionomics output associated with local malaria transmission. Decentralized program delivery and field level management will be through Environmental Health Technologists (EHTs). This cadre is responsible for the management of district based vector control activities. Fifty-five EHTs from 19 districts were recruited and have completed the training phase of the program. Participants improved their program related knowledge by 20% in exit assessments following a 5-day surveillance training (P=<0.001); (12 months after baseline training). A 13% increase (P=<0.001) in surveillance training exit assessment compared to baseline training highlights the cumulative benefits and importance of this phase of programming. Despite this cadre having no specific background in medical entomology, their management of district based vector control activities and training outcomes indicate they would be well suited to pilot the integrated entomological surveillance model. Routine entomological surveillance, leading to comprehensive spatial and temporal mapping of vector species would greatly assist the NMCC with intervention selection and targeting, whilst optimizing the use of district based human resources.

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EVALUATION OF THREE SYNTHETIC PEPTIDES FROM *GLOSSINA PALPALIS GAMBIENSIS* SALIVA AS BIOMARKER CANDIDATE OF TSETSE BITES

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Among several control strategies, anti-vectorial campaigns nowadays represent a great hope to achieve control and/or elimination of Trypanosomosis. Our study proposes a new strategy, alternative or complementary to entomological methods to assess the impact of vector control campaigns. It is based on the detection in humans of antibodies directed against *Glossina* specific salivary antigens. This evaluation provides a direct biomarker of human's exposure to tsetse bites. The main objective of this study is to improve the specificity and reproducibility of this biomarker of exposure to the bites of tsetse currently focused on whole saliva. To accomplish this purpose, we realized 2D gel electrophoresis followed up to blots with pools of human plasma exposed and unexposed to tsetse's bites. Blots alignment by the Samespots software followed by mass spectrometry analysis allowed identifying of tree specific proteins: adenosine deaminase (ADA-41KDa), Tsetse Saliva Growth Factor1 (TSGF1-56KDa), and antigen 5 (AG5-29KDa). Bioinformatic analysis using epitopes prediction softwares and Blast alignments led to target 3 sequences from these three proteins that are potential candidates of biomarker of exposure. Evaluation of each peptide was performed by indirect ELISA in a cohort composed by plasma from exposed individuals from Guinea Burkina and supposed negative controls from South-Benin and France. TSGF1 peptide allowed a suitable differentiation of populations exposed to tsetses bites (Guinea) to those who are less or no exposed (Bobo, South-Benin and Bordeaux) in contrary to peptides issue from ADA and Ag5. Results obtained with TSGF1 peptide showed that more than 60% of the Guinea population is highly exposed against only 0% and 4, 5% in South-Benin and Bordeaux respectively. In view of its antigenicity and its specificity to *Glossina*, TSGF1 is selected as the best candidate for the development of a sensitive, specific and standardized immunological biomarker.

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TOWARDS AN EFFECTIVE TRAP FOR REPLACING HUMAN BAIT IN THE SURVEILLANCE OF BLACK FLIES *(DIPTERA: SIMULIIDAE)* IN ONCHOCERCIASIS ELIMINATION PROGRAMS

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Onchocerciasis (river blindness), a debilitating vector-borne disease caused by infection with the filarial nematode, *Onchocerca volvulus*, has been targeted for global elimination by several governmental and nongovernmental health organizations. The criteria necessary to declare interruption of transmission in an area focuses on testing large numbers of the vectors (*Simulium* spp. black flies), as this method provides the most direct indication of transmission status at any given point in time. However, the collection of the necessary number of flies to demonstrate interruption represents a serious operational bottleneck. This is because the only commonly used method to collect Onchocerciasis vectors relies upon human landing collections – an inefficient and ethically unsound practice. A trap to replace human landing collections is desperately needed. To this end, field studies were carried out at sites of historic Onchocerciasis transmission in Oaxaca and Chiapas, Mexico to evaluate potential black fly traps. Seven traps were selected due their reported effectiveness for trapping black flies (*Simulium* spp.) known to transmit the parasite in these remote locations. The traps were aimed at collecting host seeking females (Silhouette-type traps, Chemical lure baited traps) or oviposition-site seeking females (Bellec plaque) of *Simulium ochraceum* s.l., the primary vector of Onchocerciasis in Mesoamerica. Only one of the seven candidate traps, a novel design dubbed the "Esperanza black window trap" showed promise as a method for replacing human landing collections in Mesoamerica. The number of *S. ochraceum* females captured by the Esperanza black window trap was significantly greater ($P < 0.05$) than the number caught by all other trap types. When baited with carbon dioxide and a commercially available human scent lure, the Esperanza black window trap caught numbers of *Simulium* females rivaling human landing collections (94% as many flies captured by human landing collections during the same time period). Our results reveal a proof of concept for moving forward with optimizing a trap to replace human landing collections in Mesoamerica. The effectiveness of the Esperanza black window trap for collecting vectors of Onchocerciasis in South America and Africa should also be evaluated.

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CAN VECTOR CONTROL AGAINST SLEEPING SICKNESS BE MADE AFFORDABLE? A FIELD TRIAL IN UGANDA USING 'TINY TARGETS'

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Human and Animal African Trypanosomiasis (HAT and AAT respectively) are transmitted by tsetse (Diptera: Glossinidae). Whereas savannah species of tsetse are the main vectors of AAT, tsetse of the riverine group are responsible for the transmission of >95% of HAT cases. Campaigns against the main vectors of AAT have been implemented since the 1930s. Conversely, efforts to tackle HAT have been based on medical interventions, largely because methods for controlling tsetse are too expensive and logistically demanding. For example, the density of insecticide-treated targets or traps required to suppress tsetse is about four times greater when targeting riverine species, compared to savannah species. In recent years, attempts to reduce the cost associated

with vector control resulted in the development of 'tiny targets' (25 cm high x 50 cm wide), 8x smaller than standard targets (~1 m2). This new technology is being tested in semi-controlled conditions in Chamaunga (Lake Victoria, Kenya), an island of about 0.5 km2 where the population of *Glossina fuscipes fuscipes* was reduced ~99% in five months with 20 targets. 'Tiny targets' are also tested in a larger field trial (~500 km2) in West Nile (northern Uganda). Targets will be deployed along the narrow linear riverine habitat only, where G. f. fuscipes are present (~15-20 targets/km). In addition to the tsetse density monitoring, the incidence of trypanosomiasis in cattle will be used as an indicator of the intervention impact. In a preliminary survey, the parasitological examination showed that 28% (169/606) of cattle were infected; the prevalence increased to 63% (185/293) with PCR. In the coming two years and together with the intervention report, the financial implications of this new technology will be explained; a reduction of about 75% in the cost is expected, compared to a similar intervention using the conventional technology, i.e. 1x1 m targets or traps.

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MYCETOMAS DIAGNOSED IN SENEGAL

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Mycetomas are inflammatory pseudotumours of subcutaneous and possibly osseous soft fabrics, generally polyfistulas with chronic mode of evolution. This study was carried out at the laboratory of parasitology and mycology of Le Dantec hospital in Dakar, Senegal, including 113 patients, from june 2008 to july 2010. Patients were from different regions in Senegal and in neighborhood countries, referred to the laboratory for mycetoma diagnosis. Among the 250 patients referred, 113 were positives after direct observation and culture corresponding to 45.2% index of infestation. The age range varies between 13 to 73 years with an average age of 33.9 years. The age bracket ranging between 20-39 years is more infected (27.34%), followed by 40-59 years (25.2%), 60 years and more (4.5%), 30-39 years (16.64%), 13-19 years (7.2%). The infection sex rate was, male: 79.6% and female: 20.4%. Infection prevalence profession dependant was found mainly in farmers and breeders with respectively: 48.7%, and 42.5%. The foot infestation is most represented with 72.5%, then leg (12.3%), knee (7.1%), scalp (2.7%), hand (1.8%). The other localizations are found with less than 1%: back, thigh, chest and ganglion inguinal. According to mycetoma agents, fungy are represented than mycetomas actinomycosic (bacterial) with respectively 70% and 30%. The species found were: *Madurella mycetomatis* (53.1%), *Actinomadura pelletieri* (23%), *Leptosphaeria senegalensis* (9.7%), *Streptomyces somaliensis* (2.6%), *Actinomadura madurae* (2.6%), *Pseudallescheria boydii* (1.8%), *Nocardia spp* (1.8%), *Scedosporium apiospermum* (0.9%), *Fusarium solani* (0.9%). We found agents of dermatophytes: *Microsporum langeronii* (1.8%), and *Trichophyton mentagrophytes* (0.9%).
This study confirms that mycetomas are endemic affections in Senegal, where it still remain a real cause of disability among population leaving in rural area.

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URINARY TRACT INFECTIONS: PREVALENCE, PATHOGENS, AND ANTIMICROBIAL SUSCEPTIBILITY PATTERNS AMONG UNDER-FIVE FEBRILE CHILDREN IN DAR ES SALAAM, TANZANIA

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Urinary tract infection is a common and important cause of morbidity in the pediatric population in developing countries. Prevalence rates of UTI in children ranges from 3.3% in the United States to 39.7% in Northwestern Tanzania. Diagnosing UTI in children is difficult to establish solely on clinical grounds, therefore in resource-limited settings most children with UTI are either misdiagnosed or given empiric treatment without laboratory confirmation of the infection. Moreover, many uropathogens are developing resistance to antibiotics recommended by WHO to treat UTI. The magnitude,etiology and antimicrobial susceptibilities of UTI in Tanzanian febrile children are currently not well defined. All published studies to date on the prevalence and etiologies of UTI in febrile children have not included a control group which is necessary to investigate false positives due to poor collection procedures or a latent period before the sample is inoculated to the agar growth media.UTI in under-five febrile Tanzanian children are under-and misdiagnosed frequently. Ail of the study is to determine the prevalence,etiology,and antimicrobial susceptibility of UTI in 2-5years febrile children attending a district hospital outpatient clinic in Dar es Salaam,Tanzania. Results will facilitate a more rational choice of antimicrobial treatments and will allow more efficient disease treatment, faster disease resolution, prevention of disease progression, and ultimately less expensive treatment regimens.

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GENETIC DIVERSITY AMONGST MENINGITIS AND BACTEREMIA CAUSING PNEUMOCOCCI FROM MALAWI

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Pneumococci are highly recombigenic nasopharyngeal commensals capable of invading a range of sterile sites, causing life threatening disease. The host and bacterial factors that determine whether a particular strain is capable of causing meningitis or bacteraemia are largely unknown. We hypothesised that there is a difference in genetic diversity between pneumococci associated with different disease outcomes.
We therefore defined the core-genomes of bacteremic and meningitic isolates in Malawi to identify key genes that are essential for invasion. 140 randomly sampled genomes (70 meningitis and 70 bacteremia isolates) were submitted to high-throughput Illumina sequencing. We clustered encoded genes using OrthoMCL to identify orthologs and define core-genes and then identified differences in the distribution of core-genes. To understand the strain structure, the phylogeny of the strains was re-constructed using a maximum likelihood approach. The core-genome of the entire pneumococcal dataset consisted of 1228 genes. Using a two-exponential model we found that meningitis-associated isolates had a larger core-genome than bacteremia-associated isolates (1338 vs. 1284; R2 99.7 and 99.8 respectively; $p< 0.001$). These highly conserved meningitis genes consisted largely of virulence factors and metabolic genes. This core genome difference is neither clonally driven nor the result of a dominant clonal-complex. HIV status did not influence genetic diversity. In conclusion, meningitis-causing pneumococcal isolates have a highly conserved complement of virulence factors and metabolic genes not present in all pneumococci isolated from blood. This genome analysis approach can be used to better understand pneumococcal biology and identify novel vaccine targets.

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UNDERSTANDING RESPIRATORY MECHANISMS IN MYCOBACTERIA

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Mycobacterium tuberculosis (Mtb) is the causative agent of tuberculosis (TB), one of the major infectious diseases, affecting one-third of the world population. Persistence of Mtb despite prolonged chemotherapy represents a major obstacle for the control of tuberculosis. Mtb is an obligate aerobe which has the ability to survive and persist for a long period of time even under conditions of low oxygen tension (hypoxic conditions). The mechanisms employed by Mtb to persist in a quiescent

state are largely unknown. Respiration is a major process through which Mtb generates ATP and intracellular concentration of ATP is 5-6 times lower in hypoxic non-replicating Mtb cells compared to aerobic replicating bacteria, making them exquisitely sensitive to any further depletion, as reported previously. Successful phase IIb studies using TMC207 has clinically validated ATP synthase as an important target, as reported previously. But, the respiratory mechanisms of mycobacteria are not thoroughly understood. The respiratory chain of mycobacteria involves many complexes namely NADH dehydrogenase, nitrate reductase, fumarate dehydrogenase, succinate dehydrogenase, cytochrome oxidase and ATP synthase. A systematic approach using biochemical assays will be employed to understand the importance of various complexes involved in respiratory mechanism. Chemical genetic and genetic approaches would also be employed to study the respiratory physiology of aerobic growing and hypoxic non growing mycobacteria. It is anticipated that important discoveries will be made in this project which will eventually buttress tuberculosis drug development in the search for novel antimycobacterial agents.

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NASAL CARRIAGE OF *STAPHYLOCOCCUS AUREUS* AND METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) AMONG HEALTH WORKERS AND INPATIENTS AT KORLE BU TEACHING HOSPITAL IN GHANA

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Nasal carriage of *Staphylococcus aureus* is a risk factor for *S. aureus* infection such as bacteraemia, surgical site infection, pneumonia, endocarditis, cellulitis and abscesses. Methicillin Resistant Staphylococcus aureus (MRSA) is a well known pathogen responsible for serious infections; its resistance to commonly used antistaphylococcal agents makes it a threat to public health. It is a major cause of hospital acquired infection worldwide, however, in information on this pathogenic organism in hospital setting here in Ghana is scarce, this study therefore determined, the nasal carriage rate of *S. aureus* and MRSA among health workers and inpatients at Korle bu Teaching hospital, the largest health care facility in Ghana with over 2,000 beds and an average admissions of 250 patient daily. Nasal swabs were obtained from health care workers (HCWs), mainly Physicians, and Nurses and inpatients at the Child health and Surgical Departments. Participant's age, sex, diagnosis, period of hospitalisation, travel record and history of antibiotic use were also obtained. .Nasal swabs were pre enriched in 6.5% NaCl Muller Hinton broth and incubated at 37°C for 24hrs and plated on blood agar. S. aureus isolates were identified by standard biochemical test. Data was analysed by Person chi-square and fisher exact test. A total of 292 nasal swabs were obtained from HCWs (131) and In- patients (161), 67 tested positive for *S. aureus*. Nasal carriage rate of *S. aureus* was found to be high in HCWs (39 (30%) compared to in-patients 28 (17%) (OR=2.25, 95%CI=1.16-4.36; p=0.0179), 14 (21%) out of the 67 S aureus isolated, 14 (20.9%) were MRSA: (HCWs:8(20.5%); In-patients:6(21.4%)); 87%, 24%, 22%, 20.9% 4.5%, 4.5%, 3%, 0%, 0%, 0% were resistant to penicillin, tetracycline, fucidin, cefoxitin, norfloxacin, erythromycin, clindamycin, gentamicin, linezolid and rifampicin respectively. In conclusion, the overall nasal carriage rate of *S. aureus* and MRSA were found to be 23% and 20.9% respectively, this call for active surveillance in the health care facility to prevent its spread.

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GENOTYPIC CHARACTERIZATION OF *STAPHYLOCOCCUS AUREUS* STRAINS ISOLATED FROM HEMODIALYSIS CATHETERS OF MEXICAN PATIENTS

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Staphylococcus aureus is an important pathogen able to produce a great number of adhesins and toxins, and to form biofilms. The icaA operon and the rbf and sarA regulator genes are involved in biofilm formation. Agr-mediated biofilm dispersion can lead to bacterial dissemination to other body sites. The aim of this work was to study the expression of icaA, rbf, sarA and agr genes and the frequency of 12 adhesin- and 14 toxin-coding genes in a group of *S. aureus* strains isolated from tunneled hemodialysis catheters. Catheters of 109 patients (48 women, 61 men; 17-77 years old) suffering chronic renal failure were analyzed. S. aureus was identified by PCR amplification of femA, femB and nuc chromosomal genes in 55 samples (50.4%). Expression of icaA, rbf, sarA and agr was determined by real time PCR. Adhesin- and toxin-coding genes were detected by PCR. All strains carrying icaA (n=53), rbf (n=22), sarA (n=41) or agr (n=55) were able to express them. Eighty two percent (n=45) of the strains were mecA+. A high proportion of the strains possessed the adhesin coding genes: sdrC (89%), sdrD (89%), sdrE (87.2%), ebps (85.4%), clfB (81.8%); the toxin coding genes hlg (92.7%), seg (92.7%) sei (85.4%), seh (78.1%). Half of the catheters analyzed were contaminated by S. aureus, most of which were methicillin resistant. A high percent of the strains expressed the virulence markers involved in biofilm formation and bacterial dispersion, and had genes coding for adhesins and toxins. This study contributes to the recognition of virulence gene prevalence among *S. aureus* strains from hemodialysis catheters.

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POINT MUTATIONS IN THE FOLP GENE PARTLY EXPLAINS SULFONAMIDE RESISTANCE WHILE OVEREXPRESSION OF THE FOLA GENE CAUSES TRIMETHOPRIM RESISTANCE IN *STREPTOCOCCUS MUTANS*

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Cotrimoxazole (trimethoprim and sulfamethoxazole) inhibits microbial dihydrofolate reductase (DHFR) and dihydropteroate synthase (DHPS). The drug is commonly used for prophylactic control of infections . However, cotrimoxazole has been reported to select for resistance in pathogenic microbes. To determine the mechanism of cotrimoxazole resistance in *Streptococcus mutans*, we analyzed the genes encoding dhfr (folA) and dhps (folP) in field bacterial isolates. Streptococci were obtained from saliva of HIV/AIDS patients taking cotrimoxazole prophylaxis in Uganda. The bacteria were tested for cotrimoxazole resistance and chromosomal DNA was extracted. Different mutant fol P genes were prepared and separately transformed into folP knock out *E. coli* cells (C600 ΔfolP). With media containing sulfamethoxazole (SMX), we assessed the growth of knockout *E. coli* cells transformed with plasmid carrying different folP gene mutations. Isolate 797 and several of its related isogenic strains were selected on medium containing trimethoprim (TMP). Differences in expression levels of the folA gene in TMP-resistant and susceptible bacteria were determined using real time PCR. While isolate 797 possessed folP mutations A46V, E80K, Q122H and S146G, as compared to control strain NN2025, combinations of these four mutations did not affect transformed knock out susceptibility to SMX. Nonetheless, TMP resistance in isolate 797 was accompanied by ten-fold increase in expression of folA gene. Conversely, isolate 8 possessed the folP mutations A37V, N172D and

R193Q in comparison to control strain UA159. Interestingly, isolate 8 folP gene not only conferred substantial resistance to SMX but also changes in any of the three amino acids of isolate 8 folP reduced SMX resistance, and removal of all three mutations totally abolished SMX resistance.

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THE POST-ENDEMIC SURVEILLANCE PROTOCOL, STRATEGY, IMPLEMENTATION AND RESULTS TO DATE IN THE NATIONAL TRACHOMA CONTROL PROGRAM IN MALI

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Since 2000, Mali has implemented the SAFE (surgery, antibiotics, facial cleanliness, and environmental improvement) strategy for trachoma control. The national objective is to eliminate trachoma as a blinding disease by 2015. In many districts, Mali has achieved the target of reducing the prevalence of trachomatous inflammation follicular (TF) to below 10% and has begun post-endemic surveillance (PES). World Health Organization recommends that sub-district level surveys take place in districts where TF among children 1 to 9 years old is less than 10%. Mali is the first countries to test these recommendations. The PES began gradually in 2011 in districts where TF prevalence had dropped below 10% after consecutive rounds of mass treatment in five regions. In each Health District (HD), 10 Health Areas (HA) were randomly selected each year, and within each HA two villages were randomly chosen for surveillance. The Ophthalmology Medical Assistant (AMO) in each HD visited these selected sentinel sites to assess TF prevalence in 50 children aged 1-9. For areas where access was not possible by motorbike or where there was no AMO, surveillance was conducted by the National Program for Blindness Prevention (PNLC). A total of 73 sentinel sites were visited in 46 HAs in 13 HDs. In 13 HDs, only two had a prevalence of TF between 5 and 10%; with all other sites having a low prevalence of TF below 5%. These visits identified 8 sites in 6 HAs with TF prevalence of 10% and above, 11 sites in 11 HAs with TF between 5 and 10%, and 54 sites in 29 HAs with TF prevalence less than 5%. This data will drive PNLC decision-making on strategic implementation of the SAFE strategy. Regarding the "A" component, six HAs and 11 villages will recommence with MDA of azithromycin and tetracycline for 3 years. The PNLC will establish PES sites in the other HDs in collaboration with its partners in trachoma control. In conclusion, the PES has allowed the PNLC to identify those remaining pockets of endemic trachoma at the HA and village level that require additional mass antibiotic treatment.

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FREQUENCY OF *BLA*$_{IMP}$, *BLA*$_{VIM}$ AND *BLA*$_{NDM}$ GENES ENCODING METALLO-ß-LACTAMASES IN CARBAPENEM-NON-SUSCEPTIBLE *ACINETOBACTER SPP* AND *PSEUDOMONAS AERUGINOSA* ISOLATES IN LIMA-PERÚ

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Carbapenemases belong to Ambler class B, called Metallo-β-lactamases, have the ability to hydrolyze a variety of β-lactam antibiotics like penicillins, cephalosporins and carbapenems. Acquired Metallo-β-lactamases have been reported mainly in *Acinetobacter spp* and *Pseudomonas aeruginosa:* they have also been reported in Enterobacteriaceae. There are different families of Metallo-β-lactamases; the IMP and VIM types are the most widely reported worldwide and the last corresponds to NDM. The present study was conducted to determinate the frequency of the epidemiological and clinical relevant genes encoding Metallo-β-lactamases in Carbapenem-non-susceptible *Acinetobacter spp* and *Pseudomonas aeruginosa* isolates in Lima-Perú. 119 *Pseudomonas aeruginosa* isolates and 20 *Acinetobacter spp* isolates were recovered from different Hospitals corresponding to community-acquired infection and nosocomial infections between july 2010 and march 2012. Susceptibility test by Disk diffusion was performed according to the Kirby-Bauer Method in order to verify the resistance pattern of each isolate. Phenotypical detection of Metallo-β-lactamases with EDTA, Ceftazidime, Imipenem and Meropenem disks was performed. There was 15 positives from 119 *Pseudomonas aeruginosa* (12.6%) and neither from *Acinetobacter spp*. Multiplex PCR for detect *bla*$_{IMP}$, *bla*$_{VIM}$ and *bla*$_{NDM}$ genes encoding Metallo-β-lactamases shows *bla*$_{IMP}$ gene in 15 *Pseudomonas aeruginosa* isolates mentioned above; they were resistant to Imipenem and Meropenem and correspond to nosocomial infections. Rapid detection of relevant genes encoding Metallo-β-lactamases is important like infection control to reduce their spread.

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FINGERPRINTING OF *MYCOBACTERIUM TUBERCULOSIS* BY RANDOM AMPLIFIED POLYMORPHIC DNA: EVIDENCE OF TUBERCULOSIS TRANSMISSION IN YAOUNDE, CAMEROON

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Although the lower-resource countries have by far the highest burden of tuberculosis, knowledge of Mycobacterium tuberculosis genetic diversity in these regions remains almost inexistent. In this study the usefulness of RAPD analysis for typing of Cameroon strains of *Mycobacterium tuberculosis* was investigated to determine by strain identification of *M. tuberculosis*, whether transmission has occurred between individuals or whether new strains are present. 93 samples of *M. tuberculosis* isolates circulating in Yaounde-Cameroon were genotyped by RAPD analysis using 10 different primers. There are three groups (I to III) of *M. tuberculosis* prevalent in Yaounde city, Cameroon. The major group III which had 72% of similarity was present and transmitted continuously. Group I and II had been eradicated. Population genetic tests revealed a basically clonal structure for this population, with small excluding rare genetic exchanges. Genetic analysis also showed polymorphism for the species *M. tuberculosis*. The prevalence of tuberculosis in Yaounde Cameroon is due to transmission rather than reactivation, but lack of efficient diagnostics also may play a role in tuberculosis transmission.

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MATERNAL COLONIZATION AND EARLY-ONSET NEONATAL SEPSIS IN DHAKA, BANGLADESH

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Globally, neonatal infections account for 900,000 annual neonatal deaths. During the first week of life, infections may cause 42% of early neonatal deaths. The modes of transmission and risk factors for early-onset neonatal sepsis remain poorly understood in developing countries. To estimate the risk of developing early-onset neonatal sepsis among newborns born to mothers with vaginal-rectal bacterial colonization compared to newborns born to non-colonized mothers in Dhaka, we conducted a prospective cohort study at a maternity center in Dhaka following 600 mother-newborn pairs from January 15, 2011 to October 31, 2011. Women with a positive bacterial vaginal culture (positive for *Staphylococcus aureus*, non-group B *Streptococcus* species, Group B *Streptococcus* (GBS), *Klebsiella pneumoniae, Escherichia coli*, *Staphylococcus, Pseudomonas*, or *Actinobacter*) or positive GBS rectal culture during labor were classified as colonized. Newborns born via vaginal delivery were followed over the first seven days of life. The primary

outcome measure was physician or community health worker diagnosis of neonatal sepsis during the first seven days following modified World Health Organization Integrated Management of Childhood Illnesses criteria. Survival analysis was conducted with nonparametric, parametric, and semiparametric models. Of the 600 mother-newborn pairs, 64 newborns (11%) were diagnosed by a physician or a community health worker with early-onset neonatal sepsis. Two hundred and ten mothers (35%) were colonized; 170 singly colonized and 40 co-colonized. The most common organisms were Non-GBS Streptococcus, S. Aureus, and *E. coli*. Newborns born to colonized mothers developed sepsis 43% faster than newborns born to non-colonized mothers (relative time=0.57, p=0.058). The risk of sepsis increased with maternal colonization, especially during the first three days of life. Newborns born to colonized mothers are at higher risk of developing sepsis compared to those newborns born to non-colonized mothers in Dhaka, Bangladesh.

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EVALUATION OF PROTECTIVE ROLE OF IMMUNOREACTIVE PROTEINS TRP19 AND TRP36 IN A MOUSE MODEL OF EHRLICHIOSIS

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Ehrlichioses are emerging diseases distributed worldwide, which affect animals and humans. Although studies have focused on understanding of the ehrlichial pathogenesis, many aspects of mechanisms of immune protection are still unclear. Several immunoreactive proteins of *Ehrlichia* have been identified based on their reactivity with immune sera, although their protective role is only starting to be understood. In this study we evaluated the protection induced by two immunoreactive proteins of *E. muris* (EM), TRP19 and TRP36, in *a* mouse model. C57BL/6 mice were inoculated intraperitoneally with recombinant TRP19 or TRP36 proteins or a combination of both followed by a second dose 60 days later. The control groups included mice receiving saline or immunization with recombinant *Chlamydia pneumoniae* OMP and an infection-control group receiving a single dose of live EM. Serum samples were collected 30 days after the second dose to evaluate antibody production. All protein-immunized groups and the EM-control group developed antigen-specific IgG antibodies. Sixty days after the booster immunization, all groups were challenged with a high dose of EM by the i.p. route. Mice were euthanized on day 10 after challenge, and the bacterial loads were determined in blood and organs by a real time-PCR targeting the *Ehrlichia dsb* gene. Mice immunized with TRP19 or TRP36 had low levels of bacterial DNA in tissues in comparison with the saline and *Chlamydia* control groups. The lower bacterial loads observed in the TRP36 and TPR19+TRP36 groups, but not in the TRP19 group, were statistically significant. The results suggest that TRP19 and TRP36 are immunogenic proteins that induce different levels of protection, which is significant for TRP36. Further studies are needed to evaluate the efficacy of using single or combination of proteins to induce better protection.

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THE IMMUNE RESPONSE IN PERIPHERAL BLOOD MONOLAYER CELLS STIMULATED WITH *TAENIA SOLIUM* AND *T. SAGINATA* ONCOSPHERE ANTIGENS *IN VITRO*

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Human cysticercosis is a parasitic disease caused by larvae of the cestode *Taenia solium*. It is acquired by the ingestion of eggs or oncospheres of *T. solium,* which after being activated in the intestine, rapidly migrate to the blood and frequently reside in the brain ocurring neurocysticercosis. On the other hand, bovine cysticercosis is caused by cestode *Taenia saginata*. When the cattle ingest eggs, the oncospheres hatch, penetrate the intestinal mucosa and migrate through of the circulation to develop cysticerci. This cystercosis is not producing in humans. The immune response when the oncosphere migrate to the blood has not been studied previously. It is important to understand the cellular response immune of oncosphere because is the first stage of attachment to intestinal cells. Therefore, the purpose of this study was to understand the relationship of the immune response between the *T. solium* and *T. saginata* oncosphere using peripheral blood mononuclear cells (PBMC). Oncosphere antigen was prepared from eggs of *T. solium* and *T. saginata.* The oncospheres were activated and sonicated to obtain the concentration of proteins. PBMC were obtained from healthy subjects (N=3). PBMC were prepared *in vitro* with 10 and 20 ug/mL of antigen and PHA (Phytohemaglutinin) as mitogen. Cells were set up at $2x10^5$ cells/well in 96-well plates at 2 and 4 days of incubation. The cytokine detection was measured by Luminex xMAP technology - Biorad. After 2 and 4 days of stimulation by *T. solium* antigen, IL-10, TNF α, IL-6 and IL-1β were induced at 2 and 4 days while IFN γ and IL-17 were induced at 4 day. *T. saginata* antigen stimulated IFN γ and IL-17 at 4 days in low concentration respect *T. solium* antigen. While IL-10, TNF α, IL-6 and IL-1β did not exhibit response. These preliminary results show the *T. saginata* oncosphere can tolerate the immune response in PBMC from healthy people but *T. solium* oncosphere induced the Th1 and Th2 response. We need to assay in patients with neurocysticercosis and increase the number of samples.

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INDIGENOUS DIAGNOSTICS AND CLINICAL PROFILE OF NEUROCYSTICERCOSIS-A TRULY NEGLECTED TROPICAL ZOONOTIC DISEASE IN INDIA

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Taeniasis/cysticercosis is a human to human infection acquired by an enteric route from carriers of intestinal *Taenia solium*, most often in areas with improper sanitation. Adult *Taenia* carried by humans release large numbers of infective eggs which are extremely contagious. Intestinal taeniasis cause few symptoms, but cysticercosis in human brain and other vital organs like eye are of concern for the respective clinical problems. It is a global problem since there is no boundary between non-endemic developed world and endemic tropical countries because of frequent immigration or travel for various purposes. We believe that the first step required to solve the burden of taeniasis/cysticercosis is by providing accurate quantification of the incidence and prevalence of neurocysticercosis at regional level. An epidemiological intervention could be lunched to interrupt the chain of transmission by: (1) Searching for treating and reporting the sources of contagion, i.e., human carriers of tapeworms; (2) Identifying and treating other exposed contacts; (3) Providing health education on parasite transmission and improvement of hygiene and sanitary conditions; and (4) Enforcing meat inspection policies and limiting the animal reservoir by treatment of pigs. Efforts are made in Latin Americas, Mexico, and Africa for its control or eradication. However, no initiative is taken in India where we know this country is also highly endemic for taeniasis/cysticercosis. Since WHO has proposed that it should be declared as an international reportable disease, new cases of neurocysticercosis should be reported by physicians or hospital administrators to their health ministries. In this proposed mission, there is need of a proper diagnostic strategy for an effective control of taeniasis/cysticercosis. Heterogeneity is marked in the clinical profile as well as imaging features of neurocysticercosis patients in India when compared with that in other reported endemic countries. Our studies have identified few clues to diagnose the asymptomatic conditions which will be helpful in mass population surveillance studies. Today there is an alarming necessity for extending hands beyond continents for a global eradication initiative.

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SERUM ANTIGEN LEVELS CORRELATE WITH LESION SIZE IN SUBARACHNOID NEUROCYSTICERCOSIS

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The larval stage of the pork tapeworm *Taenia solium* invades the human central nervous system causing neurocysticercosis. When cysts invade the subarachnoid space, particularly in the basal cisterns, they trigger a very severe inflammatory response, grows and infiltrate neighboring spaces, causing mass effects, hydrocephalus and intracranial hypertension and resulting in considerable mortality. CT or MRI imaging demonstrate the number, location, size and evolutive stage of the lesions, as well as the host´s inflammatory reaction. Serology confirms the imaging diagnosis or clarifies cases where the images are not conclusive. Antigen detection demonstrates the presence of live parasites. Patients with subarachnoid NCC (SANCC) usually have high serum antibody and antigen levels. Since antigen levels decrease faster than antibody levels, we examined a series of serum samples from patients with SANCC to evaluate whether serum antigen levels correlate with the size of subarachnoid lesions, to provide base evidence for the use of antigen detection for follow up after surgery or antiparasitic treatment. Archive imaging data was reviewed to select 105 patients with basal subarachnoid NCC. From these, 52 had an archive serum sample taken no more than 30 days before or after the corresponding CT or MRI image. Samples were processed by a monoclonal antibody (B158/B60) based antigen detection ELISA. Forty-seven (91%) tested positive. Serum antigen levels were significantly higher in patients with SANCC and hydrocephalus than in those without hydrocephalus. In the 30 patients with SANCC but no hydrocephalus, serum antigen levels were significantly correlated with the volume of the lesion (Spearman´s $r=0.723$, $p<0.05$), but no correlation existed in patients with SANCC and hydrocephalus. Our data demonstrates that serum antigen levels directly correlate with the volume of lesions in subarachnoid NCC in the absence of hydrocephalus, providing evidence for the use of this assay as a follow up tool.

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(+)-(R)- ALBENDAZOLE SULFOXIDE IS THE EFFECTIVE ENANTIOMER IN RACEMIC ALBENDAZOLE TO *TAENIA SOLIUM* CYSTS AS TESTED IN A SENSITIVE *IN VITRO* SYSTEM

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Albendazole is a methylcarbamate benzimidazole antihelmintic drug widely used in the treatment of neurocysticercosis (NCC), the brain infection with *Taenia solium* metacestodes (cysts). After ingestion, the drug is oxidized to albendazole sulfoxide (ABZSO), the main metabolite *in vivo*, and some to albendazole sulfone. ABZSO is chiral and has the greatest antiparasitic activity. This drug has been used clinically with some success, but cure rates are sub-optimal and the search for new therapeutic options is important. The effect of chirality on drug bioactivity is documented for many compounds; enantioselective distribution and differential pharmacokinetics have been reported for ABZSO enantiomers. In the present work the antiparasitic activity of racemic ABZSO and its (+)-(R)- and (-)-(S)- enantiomers, isolated by the Varicol process, on *T. solium* cysts was evaluated individually by a sensitive *in vitro* system. Parasites were isolated from naturally infected pigs, cultured and exposed to concentrations between 10 and 500 ng/ml of the racemic mixture and of each of its enantiomers; PZQ was used as anthelmintic reference drug. The activity of each compound on the cysts was then assessed by measuring changes in size, ability to evaginate after bile stimulation, secretion of alkaline phosphatase (AP) and release of an parasite antigen recognized by a monoclonal antibody. The (+)-(R)-ABZSO enantiomer was significantly more active than the (-)-(S)-ABZSO enantiomer in suppressing release of AP and antigen into the supernatant in a dose- and time- dependent manner, suggesting that most of the activity of ABZSO resides in the (+)-(R)-enantiomer, a finding that could have therapeutical implications.

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LONGEVITY AND VIABILITY OF *TAENIA SOLIUM* EGGS IN THE DIGESTIVE SYSTEM OF *AMMOPHORUS RUBRIPES* BEETLES

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Dung beetles act as intermediary host for a variety of pig's helminths and also play an important role in the transmission dynamics of the cestode family Taeniidae, in the later acting as mechanical vector. The present study evaluated the longevity and viability of *T*aenia *solium* eggs in the digestive system of *Ammophorus rubripes* beetles. Eighty beetles were infected with *T. solium* eggs. Gravid proglottides of *T. solium* were obtained from the Center for Global Health - Tumbes (Universidad Peruana Cayetano Heredia, Tumbes, Peru). The proglottides were crushed in a mortar and then mixed with cattle faeces (1 proglottid in 2gr of faeces). One gram of this mixture was placed in each polyethylene box for 24 hours, after which each group of five beetles was transferred into a new clean box. Five beetles were dissected every three days. Eggs in the intestinal system of each beetle were counted and tested for viability. *T. solium* eggs were present in the beetle´s digestive system for up to 39 days, gradually reducing in numbers and viability, which was 0 on day 36 post infection. The present work demonstrates that the eggs of *T. solium* can stay a period of time in the digestive system of the beetle *A. rubripes*, maintaining its viability. Therefore, the potential beetles act as a spreader of porcine cysticercosis. Also, this experiment can be extrapolated to other diseases in which the beetle would fulfill the same role.

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SUCCESSFUL ANTIPARASITIC TREATMENT FOR CYSTICERCOSIS IS ASSOCIATED WITH A FAST AND MARKED REDUCTION OF CIRCULATING ANTIGEN LEVELS IN A NATURALLY INFECTED PIG MODEL

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Taenia solium cysticercosis is a common parasitic infection of humans and pigs. In humans, diagnosis and post-treatment follow up are based on neuroimaging exams, still scarce and expensive in endemic areas. We evaluated the post treatment evolution of circulating parasite-specific antigen titres in 693 consecutive blood samples from 50 pigs naturally infected with *T. solium* cysticerci, which received different regimes of antiparasitic drugs (n=37, 7 groups), prednisone alone (n=5), or untreated controls (n=6). Samples were collected from baseline to week ten after treatment, when pigs were euthanized and carefully dissected at necropsy.

Antigen levels decreased proportionally to the efficacy of treatment and correlated with infection burden as found at necropsy. Antigen levels became less than five times (in a logarithmic scale) the initial value in 20/26 pigs free of cysts at necropsy, compared to 1/24 pigs with persisting viable cysts (OR 80.0 p<0.001). If antigen kinetics after treatment of infected humans are similar to those in pigs, this assay may provide a minimally invasive and economic monitoring assay to assess efficacy of antiparasitic treatment in human neurocysticercosis.

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DEVELOPMENT OF A MODIFIED ANTIGEN DETECTION ELISA FOR MONITORING NEUROCYSTICERCOSIS CASES

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Neurocysticercosis (NCC), caused by *Taenia solium*, is the leading cause of acquired epilepsy in the world. Patients with extraparenchymal NCC are especially difficult to manage and have a poor prognosis. Extraparenchymal disease frequently causes hydrocephalus and is associated with a progressive evolution and significant mortality. Antibody detection methods, which have proved to be valuable for laboratory confirmation of presumed NCC, cannot be used to monitor the success of anthelminthic treatment because antibodies to *T. solium* may persist for years after treatment. Detection of *T. solium* antigens, which would indicate the presence of live parasites, in serum or CSF may aid physicians in evaluating and monitoring patients with extraparenchymal NCC after treatment. To facilitate patient monitoring, we adapted an established method that is specific for *Taenia* antigens by incorporating a standard curve, made from a *T. crassiceps* extract, to allow day to day comparisons of test results. We also simplified the existing assay procedure by eliminating the need for trichloroacetic acid (TCA) precipitation, a step that increased the time of the assay and consumed a large quantity of specimen. We have tested 35 known positive serum specimens from patients with confirmed NCC and 107 known negative sera. We established a preliminary cutoff of 0.02 units/mL, resulting in a preliminary sensitivity of 83%, in patients with 2 or more viable cysts, and a specificity of 100%. When we tested samples in both assay formats (the original and our new format), the qualitative results were the same (positive vs. negative) for all samples examined. This modified version of the antigen detection assay for *Taenia* specific antigens may be a useful tool for monitoring patients with extraparenchymal NCC after anthelminthic treatment.

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REGULATION OF INFLAMMATORY IMMUNE RESPONSES TO PARASITE ANTIGENS IN PATIENTS WITH NEUROCYSTICERCOSIS

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Neurocysticercosis (NCC) is an infection of the central nervous system caused by the larval stage of Taenia solium and is frequently characterized by Inflammation of the brain and meninges. Calcified parenchymal cysts (PC) and subarachnoid cysts (SA) are two major clinical presentations of NCC with differing degrees of inflammation. Few studies have investigated differences in regulatory immune responses in the two forms of human disease. In this study, we compared inflammatory and regulatory immune responses to parasite antigens and a T cell mitogen (anti-CD3) in individuals with SA (n=10) and PC (n=10) with uninfected volunteers (Ctrl; n=10) using multicolor flowcytometry to analyze the frequencies (Fo) effector CD4+ and CD8+ T cells (CD4+/CD8+), natural regulatory T cells (nTreg), and myeloid and plasmacytoid dendritic cells (mDC/pDC) expressing a pro-inflammatory and regulatory markers. Upon stimulation with anti-CD3, no differences were found in the Fo of CD4+ cells expressing any of these markers, but in the CD8+ cells, the Fo of TNFα+ cells in PC (0.3±1.3%) was 30-fold lower than in Ctrl (11.4±4.6%; p<0.03). With parasite Ag stimulation, a 4-fold lower Fo of IL-17A expressing CD4+ was found in PC (0.2±0.1%) when compared to Ctrl (0.8±0.36%; p<0.05). Conversely, analysis of nTreg cell markers revealed non-statistically significant but consistent trends for higher Fo of nTreg cells expressing down-regulatory molecules (CTLA4, GITR, PD1, and TNFRII) in PC than in SA and Ctrl. Additionally, the Fo of mDC expressing PDL-1 was significantly lower in PC compared to Ctrl (p<0.05). PC also had a significantly lower Fo of proliferating CD4+ cells to anti-CD3 and parasite Ag compared to SA (p<0.05). Thus, PC infections are associated with downregulation of inflammatory molecules and upregulation of some inhibitory molecules compared to the SA and Ctrl individuals. Analysis of the Fo of multifunctional CD4+ and CD8+ cells is ongoing. From these data we conclude that the measures of immune reactivity correlate with the observed severity of inflammation in the two different forms of NCC and may help in guiding treatment targeted to different forms of disease.

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SUCCESSFUL TRANSIENT TRANSFECTION OF THE CESTODE *TAENIA CRASSICEPS*

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Neurocysticercosis is the most frequent parasite disease of the human brain. The causal agent of human and porcine cysticercosis is the larval stage of the flatworm *Taenia solium* (Cestoda). During last two decades considerable advances on the understanding of cysticercosis have been achieved using the murine model of cysticercosis, based on *T. crassiceps*, a close relative of *T. solium*. The availability of a transfection procedure will allow to increase our understanding of this parasite disease and will make possible studies on genetic manipulation. Recent reports have described methods for the stable transfections of different parasite organisms, however, in the case of cestodes, only transient transfection has been developed for *E. multilocularis*. Our objective is to develop a reproducible method for the stable transfection of *T. crassiceps*; here we describe the successful transient transfection. Larvae of *T. crassiceps* were maintained through intraperitoneal passage from mouse to mouse using 9 weeks Balb/cAnN females. For transfection, we used plasmid TOPO TA (Invitrogen) encoding GFP with a CMV promoter through direct microinjection of the larva. Localized GFP fluorescency occurs mainly on bud formations, lasting for 24-32 hrs. Western blot analysis using α-GFP specific antibody clearly showed the recognition of a protein band of 27 kDa, demonstrating the expression GFP up to 72 hrs after transfection. For the stable transfection, we are working on two directions, development of plasmid constructions for integration into the genome and isolation of *T. crassiceps* germ cells. Results from inmunolocalization studies using α-VASA primary antibody and colloidal gold as secondary antibody test, showed the presence of cytons that are positive to this germ-cell marker. Moreover, Western blot detection confirmed the VASA expression in crude extracts of *T. crassiceps*. In summary, we have developed the basic tools to attempt stable transfection of *T. crassiceps*.

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PROTEOMICS OF THE HOST-PARASITE RELATIONSHIP IN *TAENIA SOLIUM* CYSTICERCOSIS

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Cysticercosis is a parasite disease caused by the larvae of *Taenia solium* that is still prevalent in countries of Latin America, South East Asia and Africa. However, human migration has also increased its incidence in industrialized countries. Little is known about the physiopathology of this complex host-parasite relationship; nevertheless, it is known that cysticerci are able to evade and even modulate the host immune response as a way to survive in the host tissues. This study was design to analyze the proteomic patterns of both soluble and surrounding proteins of cysticerci collected from central nervous system and skeletal muscles from infected pigs to find out proteins associated with cysticercal localization and/or the intensity of the local inflammatory response. Briefly, we used sera from pigs with naturally acquired cysticercosis and a serum from a non infected animal for comparison. Tissue samples and vesicular fluids of cysticerci dissected from muscle or brain of infected pigs were also obtained. The sera and the cyst's tissues and vesicular fluids were separated by 2D-PAGE. The sera from infected and non infected pigs showed an average of 126 and 116 protein spots, respectively. About half of these proteins can be clearly identified using different 2D protein maps. Seventy two spots were not shared between both sera; 26 were exclusive of the infected animals. After analysis of the 26 spots through MALDI-TOF, 8 proteins were identified including clusterin, serpin A3, IgM and Apo-A1. Numbers of protein spots in the tissue samples and in the vesicular fluids of cysticerci were more variable: cysts dissected from the central nervous system of the pigs showed 301-340 protein spots in crude extracts of parasite tissue and 250-320 in the vesicular fluids, whereas those dissected from skeletal showed 270-310 for the tissue and 250-270 for the fluid. We are currently working in the identification of these protein spots. As an example, at least 6 proteins are of host origin, including porcine serum albumin and IgG, accounting for about 5 % of the total protein

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CO-CIRCULATION OF MULTIPLE SEROTYPES OF DENGUE VIRUS IN SOMALIA, 2011

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Dengue (DEN) is an arthropod-borne acute infectious disease caused by the enveloped, single-stranded RNA dengue virus of the family Flaviviridae. There are four serotypes which share genetic and antigenic features, but infection with one serotype does not provide long-term protection against the other serotypes. In June 2011 an outbreak of acute dengue-like febrile illness was reported in Mogadishu, Somalia. Serum samples were collected and sent to the KEMRI/Walter Reed Viral Hemorrhagic Fever laboratory for diagnosis. A molecular analysis to determine the circulating dengue virus serotypes and the extent to which the Somalia population had been exposed to multiple dengue virus serotypes was done using Reverse transcriptase Polymerase Chain Reaction (RT-PCR) and sequencing analysis. RNA was isolated from 16 serum samples using the QIAamp viral RNA mini kit (Qiagen, Germany) according to the manufacturer's protocol. An RT-PCR assay was performed using dengue consensus and serotype specific primers to distinguish between the four DEN serotypes. Amplicons of six of the positive samples were further sequenced to confirm the results and phylogenetic analysis done to establish the evolutionary relationship of these DEN viruses compared to others obtained from the genbank database.Eleven out of sixteen (69%) samples were positive for Dengue virus using Dengue consensus primers. Out of the positive samples, six (38%) were DEN-1; three (19%) were DEN-2 and two (12.5%) were DEN-3. The six positives (four DEN-1; one DEN-2 and one DEN-3) that were sequenced and compared with other available sequences in the genbank database showed that DEN-1 virus from Somalia was closely related to the Thailand, Djibouti and China strains. DEN-2 virus clustered with strains from Indonesia, Burkina Faso, China and Australia while DEN-3 virus was most similar to the China and India strains.Three DEN serotypes i.e. DEN-1, DEN-2 and DEN-3 were found to be co-circulating in Somalia during one outbreak although no individual had exposure to more than one dengue virus serotype.

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GENETIC AND BIOLOGICAL CHARACTERISTICS OF DENGUE VIRUSES FROM INDONESIA

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Dengue fever is still the most important mosquito-borne viral disease in Indonesia. The pathogenesis of dengue disease is complex and involved various factors, among others are the host's immune response and viral genetic factors. Dengue virus (DENV) genetic diversity is represented by the presence of four DENV serotypes (DENV-1; -2; -3; -4). Within serotypes, viruses can be classified into many genotypes based on nucleotide sequence of the genome. It has been reported that some DENV genotypes possess unique geographical distributions as well as differ in both fitness and virulence. Dengue is endemic in Indonesia, however, currently little information is available on the serotypes and genotypes of the viruses circulating in cities across Indonesia. To gain information on the dynamics of dengue disease in Indonesia, we performed molecular genotyping of viruses isolated in nine cities in the country. Genotyping was performed using nucleotide sequence of whole genome or DENV E protein. All four DENV serotypes are circulating in the country with some serotypes are predominant in particular cities. Comparison with historical genotype data in Indonesia revealed the introduction of new genotypes and thus co-circulation with existing genotypes, e.g. Genotype I and IV of DENV-1 and Genotypes I and V of DENV-3. With the diverse genetic characteristic of Indonesian DENV isolates, we sought to understand the biological characteristic of the viruses by assessing the growth kinetics of viruses representing each serotype/genotype. We observe the relatively higher rate of replication of Genotype I compared to Genotype IV of DENV-1 viruses. To further determine whether particular serotypes/genotypes of DENV induced different host's immunological response, we assessed the expression profiles of 26 human cytokines/chemokines in an *in vitro* DENV infection model. Equal levels of cytokines/chemokines expression were induced upon infection with all serotypes/genotypes; however, some DENV strains exhibited lower induction levels compared to other. We report here the genetic and biological characteristics of DENV in Indonesia.

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CD8 T CELL RESPONSES TO A HIGHLY CONSERVED HLA-B57 RESTRICTED DENGUE VIRUS EPITOPE

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The four serotypes of Dengue virus (DENV 1-4) are the most common cause of viral hemorrhagic fever worldwide. Epidemiological evidence suggests that severe disease is associated with a variety of factors including secondary infection by a DENV serotype different from that of the primary infection and the HLA haplotype of the individual. CD8 T cells are hypothesized to play an immunopathological role in secondary heterologous DENV infection. We characterized the CD8+ T cell response to a very highly conserved HLA-B57-restricted epitope on the DENV NS1 protein in PBMC from naturally-infected donors. Epitope-specific CD8+ T cells were studied directly *ex vivo*, and at the clonal level, using tetramer staining, and cytotoxicity assays. T cell lines lysed target cells expressing the DENV NS1 protein or a minimal 9 mer peptide identified using overlapping peptide pools. T cell lines were also able to lyse DENV-infected dendritic cells. Preliminary data indicate detectable frequencies of tetramer+ T cells in PBMC of Thai children during acute DENV infection and in convalescence. HLA B57 restricted T cells expressed an activated phenotype during and after the febrile phase in PBMC of patients with both mild and severe disease supporting the potential for them to contribute to protection from severe dengue disease.

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A NOVEL SMALL-MOLECULE INHIBITOR OF DENGUE VIRUS TARGETING THE NS3 HELICASE

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Dengue virus infects 50 to 100 million people worldwide each year and has become endemic in most tropical and sub-tropical countries. The number of dengue virus infections has grown significantly over the last few decades, causing dengue fever to become a major threat to the global community. Awareness and funding of dengue research has risen greatly, and while there are several promising vaccines in clinical trials, there are currently no approved vaccines or therapeutics available. A safe, orally effective therapeutic drug to treat dengue fever is still greatly needed. SIGA has identified a novel small-molecule inhibitor of dengue virus that is potent against all four serotypes of dengue virus *in vitro*. The compound is not cytotoxic in multiple cell lines, non-mutagenic, and most importantly has shown proof-of-concept efficacy in a non-lethal murine model by intraperitoneal injection. By reverse engineering drug resistance-inferring mutations into wild-type dengue virus, we have determined the compound targets the NS3 helicase domain of dengue virus. A molecular beacon-based helicase assay and an ATP hydrolysis assay have confirmed the NS3 helicase as the compound target. The mechanism of action of our compound is unique, making it an attractive new prospect in the field of dengue therapeutics.

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FIRST PHYLOGENETIC REPORT OF ALL FOUR DENGUE VIRUS SEROTYPES FROM NEPAL

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Dengue viruses (DENV) are expanding in geographical distribution and have complex dynamics. No information on dengue molecular epidemiology is available from Nepal, though the viruses were first described in the country in 2006. We report the first phylogenetics of all four serotypes of indigenous dengue virus from Nepal based on envelop (E) gene sequences. Samples were collected during the 2010 outbreak in central and western Nepal; 36 had E gene amplification (either directly from viral RNA in serum or from virus strains isolated in C6/36 cells). Complete nucleotide sequences of E gene were determined and analyzed along with the global strains archived from GenBank to regenerate phylogenetic trees using maximum-likelihood method. We were able to obtain complete E gene sequences of all four DENV serotypes. Nepal DENV-1 strains were grouped in genotype-V forming 2 distinct clades. DENV-2, 3, and 4 strains were clustered into cosmopolitan genotype, genotype-III, and genotype-IIB, respectively. The phylogenetic findings corroborate that the majority of dengue strains were closely related to strains from South Asian countries (particularly India) and recent Singapore strains. Although similar strains were found in circulation in the South Asian countries, Indian strains are the most plausible origin of Nepal dengue viruses considering the geographical proximity. This sequence database helps in understanding the dynamics of dengue evolution and sheds light into the potential of future outbreaks in the region and their morbidity.

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DISEASE ECOLOGY AT THE INTERSECTION OF MAN AND MOSQUITO

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The past 30 years has witnessed a dramatic re-emergence of epidemic vector-borne diseases throughout much of the world. The principal drivers of this resurgence have been increased population growth, international travel and trade, changing agricultural practices, and increased urbanization. The local patterns of transmission for a vector-borne disease are driven by both movement of the host and vector, with overlap in space and time of the host and vector leading to a potential transmission event. Although little work exists on contrasting the disease dynamics of vector-borne pathogens such as malaria and dengue, the two infections exhibit very different outbreak patterns, with dengue spreading rapidly in explosive epidemics and malaria spreading in a more clustered way across a population. Using malaria and dengue spread as examples, we hypothesize that the differing disease dynamics are the result of dissimilar contact structures for the two diseases. Using a contact network framework, we characterize the patterns of local host movement as they overlap with a sessile, day-biting vector (such as *Aedes aegypti*) versus a mobile, nocturnal vector (such as Anopholes gambiae). This unique perspective on vector-borne disease transmission highlights important differences in mosquito-borne disease dynamics.

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PLATELETS ARE ACTIVATED IN ACUTE DENGUE INFECTION

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Dengue virus infection often presents as a self-limiting febrile illness, but a proportion of dengue patients develops severe complications around the time of defervescence. Severe dengue virus infection is characterized by bleeding and plasma leakage, which can lead to life-threatening shock. The pathogenesis of severe dengue infection remains largely unknown. Thrombocytopenia is a hallmark of acute dengue. Platelets are not only key cells in hemostasis, but are increasingly recognized to play an important role in immunity and maintenance of vascular integrity. The fact that bleeding manifestations and plasma leakage in dengue occur with platelet counts well above the limit for spontaneous bleeding, led us to hypothesize that acute dengue is not only associated with thrombocytopenia, but also with disturbed platelet function (thrombocytopathy) We therefore studied the presence of platelet activation and secondary platelet exhaustion in patients with acute dengue in Bandung, Indonesia. In a prospective cohort study involving adult patients with acute dengue in Bandung, Indonesia, we determined platelet activation and the sensitivity to activation using a newly developed flow cytometry assay. Baseline membrane expression of the platelet activation markers CD62P (P-selectin), CD63 (lysosomal marker) and the activated GPIIb/IIIa receptor (fibrin receptor) and the change in CD62P expression to increasing concentrations of the platelet activator TRAP (thrombin receptor agonist) were determined in the acute phase (febrile phase or the critical phase within 48 hours after defervescence), the early recovery phase (clinical and laboratory improvement) and the convalescent phase (fully recovered) of dengue infection. Seventy patients were included from March 2011 - March 2012. In the acute (febrile and critical) phase, more platelets expressed platelet activation markers as compared to the convalescent phase. In the early recovery phase we observed a reduced platelet activation response to TRAP compared to the convalescent phase. Acute dengue is not only associated with thrombocytopenia, but also pronounced platelet activation with secondary platelet exhaustion. This may contribute to the bleeding complications and plasma leakage. Prevention of excessive platelet activation may prove to be an effective adjunctive therapy in prevention of the complication of dengue.

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EFFECTS OF DENGUE VIRUS INFECTION ON *AEDES AEGYPTI* BEHAVIOR

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Dengue virus (DEN) is an important arthropod-borne virus (arbovirus) infecting humans. Infection with any of the four serotypes of DEN can manifest as asymptomatic infection, mild dengue fever, or life-threatening dengue hemorrhagic fever and dengue shock syndrome. The *Aedes aegypti* mosquito is the primary vector for DEN transmission acquiring the virus from an infectious blood meal. Once ingested, the virus infects and replicates in the mosquito midgut epithelial cells, from where it subsequently spreads to the other parts of the insect body including the brain. Dengue infection has been shown to alter the feeding behavior and locomotor activity in *Ae. aegypti* mosquito. Since most mosquito behaviors are determined by environmental odorant stimuli that are perceived by various neurons and processed in the brain, it is expected dengue virus infection will also alter other behavioral responses of *Ae. aegypti* females to standard chemical products designed to prevent human-vector contact. Here we describe the use of a laboratory assay to quantify the contact irritancy response of *Ae. aegypti* female test populations from Thailand at various stages of DEN infection dissemination to the standard topical repellent DEET. This information is vital to our understanding not only of the effectiveness of a personal protective product for prevention of dengue virus transmission during dengue epidemics but also in describing the underlying mechanism of action of virus infection on the host-seeking response overall.

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RE-EMERGENCE OF DENGUE FEVER OUTBREAK WITH CO-CIRCULATION OF TWO SEROTYPES IN LIMA, PERU - APRIL 2012

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Dengue fever is the arboviral disease with the most significant impact in Public Health in Peru. In Lima the largest outbreak ever recorded occurred in 2005 with at least 860 cases with the presence of a single circulating serotype. The epidemic potential for dengue transmission in Lima has spread alarmingly in the last five years due to migration from endemic areas as the jungle and northern of Peru. We investigated this dengue outbreak in northern Lima to determine their epidemiological, clinical and circulation of serotypes. We performed Cross sectional study. We included patients reported in the Epidemiological Surveillance System. Was defined as "Suspect case" any person that lives in Lima with fever for 2 - 7 days and two or more manifestations of the following symptoms: headache, retrorobital, myalgia, arthralgia and rash. Data were recorded on sheets of epidemiological reporting. All serum samples were tested for anti-dengue IgM and IgG antibodies by ELISA, NS1 antigen and PCR. Until march 31, 2012 Of 407 serum samples tested of suspected cases, 121 (37,7%) were positive for dengue virus specific IgM antibodies, PCR y NS1 antigen. Most cases (42%) were adults between 20 and 50 years of age. The median age was 29 years. The most frequent symptoms were fever (98,8,%), headache (86,5%), myalgia (73,6%) and arthralgia (72%). The outbreak investigation revealed a cluster of co-circulation of two serotypes: DEN-1 (85,7%) and DEN-3 (14,3%) located in one district called "Stone Bridge". However, it was observed that the notice of increase over 60 years and under 5 years, which was associated with the presence of warning signs such as vomiting blood (35%), fainting (33%), chest pain (25%) and hepatomegaly (23%).In this area the attack rate was 2.4 cases per 1000 inhabitants and there are areas favorable for breeding of the vector and areas of high migration of people from endemic areas of dengue. The outbreak investigation confirmed the presence of dengue as an emerging public health in Lima identifying the co-circulation of two serotypes that indicates the probability of development more severe clinical manifestations of dengue. It is important to develop health services for the care of patients and prevent complications of deaths from dengue fever in Lima.

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DISCOVERY AND VALIDATION OF PROGNOSTIC BIOMARKERS FOR SEVERE DENGUE BY PROTEOMIC SCREENING

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Dengue is the most important viral vector-borne disease with more than 2.5 billion people at risk for dengue virus infection in over 100 tropical and sub-tropical countries. At least 500,000 people are hospitalized annually for dengue hemorrhagic fever (DHF), a more severe form of the disease, with fatality rates exceeding 5% without appropriate treatment. The onset of DHF occurs after the patient's fever subsides and the patient appears be recovering. Current diagnostic methods cannot predict which dengue patients develop DHF. A prognostic diagnostic test that identifies patients at risk for developing DHF could significantly reduce dengue mortality

and morbidity. We used surface enhanced laser desorption/ ionization time of flight (SELDI TOF/TOF) mass spectrometry to identify unique host biomarkers in patients with severe dengue. SELDI TOF/TOF assesses differences in proteins expression in healthy and diseased patients. In order to determine if there are unique biomarkers that can differentiate between dengue fever (DF) and DHF, we developed a panel of serum specimens that included patients with DHF, uncomplicated DF, other febrile illnesses, and healthy persons. These samples were analyzed using SELDI-TOF/TOF to identify variations in biomarkers. In addition, we tested serum from laboratory-confirmed cases of DENV serotypes 1-4 to determine if there was serotype-specific variation in biomarkers. Candidate biomarkers were further characterized by two-dimensional gel electrophoresis and mass spectrometry. We identified 25 candidate biomarkers which could distinguish between DF and DHF and found no serotype-specific variation in DF biomarkers. To validate the most promising candidate biomarker vitronectin, we evaluated serum concentrations via ELISA. Vitronectin was found at significantly lower levels in serum from DHF cases compared to DF cases. The use of SELDI TOF/TOF screening for unique host biomarkers successfully identified vitronectin as a candidate. Vitronectin has the potential to differentiate between DF and DHF; with a significant reduction in DHF cases compared to DF. Further studies to determine the dynamics of this biomarker over the progression of DF to DHF will determine its utility as a prognostic marker for severe disease.

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USE OF SMALL BLOOD VOLUMES TO ANALYZE CELLULAR RESPONSES INDUCED BY SANOFI PASTEUR TETRAVALENT DENGUE VACCINE

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Assays using limited amounts of blood are needed to monitor cellular immunity after vaccination with the tetravalent CYD dengue vaccine, based on the YF 17D virus vaccine. We developed a whole blood ICS assay using only 3 mL of blood, in parallel with a multiplex assay using PBMCs also isolated from a limited amount of blood. Responses were analyzed at different time points before and after 3-dose vaccination in a subset of 40 adolescents and 40 adults enrolled in a phase II trial in Singapore (ClinicalTrial.gov NCT NCT00880893).Vaccination induced a CYD-specific Th1/Tc1 cellular response in all participants, characterized by predominance of IFN-γ over TNF-α secretion, associated with low level IL-13 secretion in multiplex analysis of PBMC supernatants after restimulation with each CYD vaccine virus. Responses against serotype 4 predominated after the first vaccination, and were more balanced after the third vaccination. DENV NS3-specific responses with a CD4/TNFα/IFNγ positive profile were detected in vaccinated adults, both before and after vaccination, whereas YF-17D NS3-specific responses with a CD8/IFNγ profile were detected in all participants, but only after vaccination. This suggests that natural infection induced DEN NS3-specific CD4/IFNγ/TNFα responses, whereas vaccination induced YF-17D-specific CD8/IFNγ responses. One year after the third vaccination the cellular response profile remained unchanged. NS3-specific responses were stable, and serotype-specific cellular responses decreased slightly in both age groups. Our findings confirm previous clinical trial observations regarding both the nature and specificity of cellular responses induced by the CYD tetravalent dengue vaccine, and for the first time demonstrate the persistence of cellular responses after one year. We also established the feasibility of analyzing CMI with small blood samples, facilitating similar analysis in future pediatric trials.

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A NOVEL SYSTEM TO PRODUCE SAFE, EFFECTIVE AND COST-EFFICIENT DENGUE VACCINES AS DEMONSTRATED IN THE AFRICAN GREEN MONKEY

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Arbovax employs an innovative technology (Patent No. 6,589,533) to develop safe and effective live-virus vaccines coupled with a low-cost system of manufacture. The first target is Dengue virus (DV), a mosquito-borne member of the Flavivirus family, which has four serologically distinct serotypes (DV1-4). Upwards of 100 million people are at risk for dengue infection each year, and with the increasing global spread of its mosquito vectors, including *Aedes albopictus*, the Asian tiger mosquito, this number is poised to dramatically increase. Currently no vaccine or therapeutic exists to counter DV. Arbovax has created a strategy based on the straightforward concept of developing stable mutations of arboviruses that can replicate successfully in insect cells but grow poorly in mammalian cells, thus creating host-range mutant viruses. The immunogenicity and safety of three novel host-range DV vaccines (DV2ΔLIG, DV2ΔGVII and DV2G460P) containing deletions in the transmembrane domain (TMD) of Dengue virus serotype-2 (DV2) E glycoprotein were evaluated in African green monkeys. These vaccines have a shorter TMD that is capable of functionally spanning an insect but not a mammalian cell membrane resulting in production of mutants that have reduced infectivity in mammalian hosts but efficient growth in insect cells. Groups of 4 monkeys received one dose each of test vaccine candidate. No boost was administered. After immunization, the level of viremia produced by each vaccine was determined by infectious center assay (ICA). Vaccine recipient immune response to WT DV2 challenge was measured on Day 57 by ELISA and PRNT. Protection was assessed by ICA. Two vaccines, DV2ΔGVII and DV2G460P, generated neutralizing antibody in the range of 700- 900 $PRNT_{50}$. All three vaccine strains decreased the length of viremia by at least 2 days. No safety concerns were identified.

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EVALUATION OF CONDITIONS TO ENHANCE DETECTION OF DENGUE VIRUS 4 NEUTRALIZING ANTIBODIES

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Epidemiological studies of dengue are complicated by extensive antigenic cross-reaction among the four dengue virus serotypes (DENV-1 through DENV-4), particularly in individuals who have been infected by multiple serotypes. Plaque reduction neutralization tests (PRNTs) are among the most specific and oft-used tests to assess serotype-specific antibody status, yet the effects of varied experimental conditions on assay sensitivity is rarely evaluated. To address this limitation, we utilized samples from a dengue cohort study conducted in Iquitos, Peru, during a period of intense DENV-4 transmission. We identified symptomatic dengue cases through community-based surveillance and monitored changes in serostatus through serum samples collected twice yearly. Our standard assay procedures (Asian strain of DENV-4 [1036] as test virus in BHK-21 [baby hamster kidney] target cells) resulted in high specificity (>99%) but low sensitivity (<70%). To identify assay modifications that improve test sensitivity, we evaluated serial samples from 15 patients with confirmed acute DENV-4 infection but negative PRNT results. We varied test virus strain, test virus production conditions, and target cells used in the assay. For these samples, we observed only a modest increase (8%) in sensitivity when using Peruvian DENV-4 test strains and a marked increase (50%) in sensitivity when using rhesus monkey kidney LLCMK2 target cells, independent of test virus. The enhanced sensitivity in LLCMK2 cells was most apparent at earlier time points post infection. Our data underscore

the need to evaluate various PRNT conditions with well-characterized sera, as assay conditions could have a profound impact on seroprevalence surveys or studies of vaccine efficacy.

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EVALUATION OF THE DIAGNOSTIC CAPACITY OF THE TRADITIONAL AND REVISED WHO DENGUE CASE DEFINITIONS

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Dengue, a mosquito-borne viral illness, is a major public health problem worldwide, and its incidence continues to increase. In 2009, the World Health Organization (WHO) published new guidelines that included a revision of the dengue case definition. The revised case definition relies mostly on signs (nausea/vomiting, rash, positive torniquet test, leukopenia, signs of alarm) rather than on symptoms (headache, retro-orbital pain, myalgias, arthralgias), which makes it more applicable to young children. We evaluated the diagnostic capacity of both WHO case definitions in two prospective studies of dengue in Managua, Nicaragua. In the first study, we collected information from 1,160 participants recruited at the National Pediatric Reference Hospital over the past 7 years, of which 723 were laboratory-confirmed dengue cases. In the second study, a pediatric cohort study, we included data from 3,407 suspected dengue cases over the past 8 years, of which 476 were laboratory-confirmed. In the hospital study, the traditional case definition yielded 96.7% sensitivity and 21.9% specificity, whereas the revised case definition had higher sensitivity (99.9%, $p<0.001$) but lower specificity (3.0%, $p<0.001$). In the cohort study, the traditional definition had 89.3% sensitivity and 43.1% specificity, while the revised definition had similar sensitivity (91.2%, $p=0.872$) and higher specificity (46.5%, $p<0.001$). Notably, both case definitions had higher specificity in the cohort study compared to the hospital study. Reasons underlying this difference are under investigation, but earlier presentation of patients to the health center in the cohort study may partially account for it. We then evaluated the performance of two diagnostic models based on the list of signs/symptoms included in each case definition by analyzing the effect of the addition of increasing numbers of signs/symptoms on the sensitivity and specificity of case capture. The receiver operating characteristic (ROC) analysis showed no significant differences between the two models in the hospital study, but displayed a slightly better performance for the revised model (Area Under the Curve 0.77 vs 0.83 for the traditional vs revised definition, $p<0.001$). Taken together, our results indicate that both case definitions have similar capacity to diagnose dengue. Owing to their high sensitivity and low specificity, they should be primarily used for screening purposes.

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COMPREHENSIVE MUTAGENESIS OF PRM/E TO IDENTIFY NEUTRALIZING AND ENHANCING EPITOPES ON DENGUE VIRUS ENVELOPE PROTEIN

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To obtain anti-DENV Envelope (prM/E) monoclonal antibody (MAb) epitope maps at the resolution of individual amino acids, we individually mutated nearly all residues of DENV3 (CH53489 strain) and DENV4 (TVP360 strain) Envelope proteins (nearly 2,000 mutations in total), expressed each mutant in human cells, and analyzed them for effects on antibody reactivity and viral infectivity. This 'Shotgun Mutagenesis' approach offers the capability of mapping both linear and conformational epitopes, even for structurally complex proteins such as oligomeric and glycosylated Envelope proteins. The neutralizing human anti-DENV MAbs used in our studies were derived from infected patient B-cells, so represent a significant protective response of the human immune system. Critical amino acids required for the binding of each MAb were identified and visualized on the prM/E protein structure. The molecular and functional mechanisms by which MAb-epitope interactions contribute to the humoral immune response were characterized by measuring viral neutralization and antibody-dependent enhancement titers using DENV reporter virus particles (RVPs). We also determined the binding affinity and kinetics of these MAbs to intact DENV virions on a biosensor. Our goal is to map epitopes on DENV prM/E, determine their role in viral protection and pathogenesis, and how they relate to protein function. We expect that this approach will help define the range of immunodominant structures on DENV prM/E and identify novel neutralizing antibody epitopes that can be used for the development of improved therapeutics, diagnostics, and vaccine candidates.

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PRIMARY DENGUE HEMORRHAGIC FEVER (DHF) CAUSED BY DENV-1 INFECTION

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Dengue is the most important mosquito-borne viral disease of humans, with an estimated 100-200 million infections and 2 million cases of dengue hemorrhagic fever (DHF) annually. The risk of developing severe disease is influenced by viral and host factors, which are not fully understood. Dengue is reportedly the second most common cause of fever in travelers returning from developing countries. We report 3 cases of primary DENV-1 in a group of 6 travelers returning from Subic Bay and Manila, Philippines. The patients were previously healthy white females, aged 24-26, residing in Singapore. All were clinically diagnosed with dengue after developing fever, body aches, retro-orbital pain, nausea and loss of appetite 1-5 days after returning to Singapore. All 3 patients showed signs of vascular leak, mainly edema, and 2 required hospitalization for intravenous fluids. One hospitalized patient had a platelet count < 100x10^9/L and the other had nosebleeds and a platelet count of 122x10^9/L. The non-hospitalized patient met criteria for grade I DHF with petechiae, hemoconcentration and a platelet count < 100x10^9/L. All recovered uneventfully. Blood samples were obtained from 2 of the 3 other travelers of the group who reported no illness; both tested negative for IgM antibodies, ruling out asymptomatic infection. Assuming an incubation period of 4-7 days, the patients were likely infected in Subic Bay. Acute blood samples were available for 2 patients and DENV-1 was detected by virus isolation and real-time PCR. The full genome sequences of the two viruses were 100% identical, suggesting that both patients were likely infected by the same mosquito. Plaque reduction neutralization test on convalescent sera confirmed that all 3 patients experienced primary DENV-1 infections. Interestingly, in an earlier report of two Swedish tourists with confirmed primary DHF associated with DENV-1 infection in 1990, the patients were also likely infected by one mosquito. Our report highlights the potential for a relatively severe course of illness in primary DENV-1 infections.

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IDENTIFYING THE GENETIC DETERMINANTS OF DENV-3 INFECTION OF THE MOSQUITO VECTOR *AEDES AEGYPTI*

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The emergence and spread of novel dengue virus (DENV) lineages that are associated with severe dengue disease contribute significantly to the ongoing global dengue virus pandemic. In particular, the emergence of a lineage of DENV-3 in Sri Lanka, and its competitive displacement of an endemic lineage associated with mild disease, coincided with an escalating outbreak of dengue hemorrhagic fever in that country. Results of previously reported studies have demonstrated significant differences in the replication and dissemination of the Sri Lankan DENV-3 lineages associated with mild and severe disease in the mosquito vector *Aedes aegypti*. The study's most important finding is that the invasive, severe disease associated lineage both replicates to high titers within *A. aegypti* and disseminates more efficiently in the vector than the native lineage associated with mild disease. However, the viral genetic determinants of this differential dissemination have not been determined. Using representative viruses from the Hanley *et al.* study and a DENV-3 clone platform, we have initiated studies to identify the viral genetic determinants of DENV-3 differences in *A. aegypti* infectivity. Using a recently described four fragment DEN-3 infectious clone that allows re-shuffling of genomic fragments between different parent virus backgrounds, we will address three aims: 1) determine the infection and growth characteristics of representative native, invasive and chimeric viruses in mammalian and insect cell lines, 2) determine the relative infectivity of native, invasive and chimeric viruses in *A. aegypti,* and 3) determine the specific genetic mutations leading to differential replication and dissemination in *A. aegypti*. Here we present results on the initial construction of the clones and characterization of the representative viruses and clones in Vero and C6/36 cells and *A. aegypti* mosquitos. This study will lay the foundation for future research investigating the molecular mechanisms governing DENV replication and dissemination in insect vectors.

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DENGUE HEMORRHAGIC FEVER-ASSOCIATED IMMUNOMEDIATORS INDUCED VIA MATURATION OF DENGUE VIRUS NS4B PROTEIN IN MONOCYTES MODULATE ENDOTHELIAL CELL ADHESION MOLECULES AND PERMEABILITY

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Dengue virus (DENV) causes a tremendous disease burden in Asia and Pacific countries where the *Aedes* mosquito vectors thrive. Dengue outbreak threats also loom in the United States along the Texas-Mexico border and in Florida and Hawaii. Although most DENV infections are asymptomatic or result in mild dengue fever (DF), some cases progress to dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Understanding causation of DHF/DSS progression may prove useful for the development of currently unavailable DENV vaccines and antiviral therapies. Our research approach adopts a two cell-type *in-vitro* system to delineate DENV proteins responsible for inducing immunomediators associated with increased permeability and DHF/DSS. We previously demonstrated that DENV nonstructural 4B protein (NS4B) induced DHF-associated mediators in THP-1 monocytes and cleavage of the NS4AB polyprotein by the viral protease NS2B3, significantly increased mediator production to levels found after DENV infection. In this report using a primary human microvascular endothelial cells (HMVEC) transwell permeability model and a HMVEC monolayer, we demonstrate that the mediators secreted in the supernatants of DENV-infected monocytes increase HMVEC permeability and expression of endothelial cell adhesion molecules; maturation of NS4B via cleavage of 2KNS4B is sufficient to initiate HMVEC alterations which appear to be synergistically induced by TNFα and IL-8. These data suggest that therapies targeting the maturation steps of NS4B, particularly 2KNS4B processing, may reduce DHF-associated mediator levels and possibly reduce overall morbidity and mortality. Alternatively, TNFα inhibitors commercially available for other chronic diseases may prove to be a valid intervention strategy during the later stages of infection. Developing novel strategies to prevent DENV disease progression is a top priority in DENV research; our work may impact the field by leading to the basic knowledge required to pursue valid therapeutic strategies.

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SEROPREVALENCE OF DENGUE IN AMERICAN SAMOA, 2010

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Since the 1970s, regular dengue epidemics have caused considerable morbidity in the Pacific region. In 2009, an epidemic year, dengue incidence in American Samoa reached 644 cases per 100,000 population and decreased to 77/100,000 in 2010. However, human surveillance is limited and the true impact of dengue is unknown. A cross-sectional seroprevalence study was conducted from May to July 2010 with the primary aims of identifying risk factors for human leptospirosis, and providing an evidence base and tools to direct public health interventions in American Samoa. During the study, investigators encountered community concern about dengue and were asked by health authorities to utilize the collected serum for a dengue seroprevalence study. In October 2011, dengue serology was performed on blood samples from 794 participants aged 18-87 years at the Australian Army Malaria Institute (Brisbane, Australia). Samples were screened for the presence of dengue virus antibodies using PanBio Dengue IgG indirect enzyme-linked immunosorbent assay (ELISA) kits (Inverness Medical Innovations, Brisbane, Australia) following manufacturer recommendations and protocols. Overall, 760 of 794 (95.6%) study participants tested positive for dengue IgG antibodies, demonstrating almost universal exposure of adults in American Samoa to dengue. This is the first population-based dengue seroprevalence study undertaken in American Samoa and the third highest dengue seropositivity rate reported in the world, behind Jamaica (100%) and the Dominican Republic (97.9%). Timely public health action is required to control dengue transmission, reduce dengue morbidity and minimize the risk of dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS) in American Samoa. Of foremost importance are the development of an active surveillance system, establishment of laboratory facilities for dengue serological testing, and implementation of a comprehensive prevention and control program.

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FULL-GENOME PHYLOGEOGRAPHIC ANALYSIS REVEALS MULTIPLE ORIGINS OF RECENT DENV-4 OUTBREAKS IN BRAZIL

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Dengue virus 4 (DENV-4) reemerged in Roraima state, Northern Brazil, 28 years after it was last detected in the country in 1982. Full-length sequences were obtained for sixteen DENV-4 isolates from Roraima, Amazonas and Pará states (Northern region), and Bahia state (Northeast region) during the 2010-2011 dengue seasons, as well as from a Brazilian isolate from the Roraima epidemic reported in 1982, to study the origin and evolution of this reemergence. Spatiotemporal dynamics of DENV-4 introductions in Brazil using Bayesian phylogeographic analyses were applied to the envelope genes and full genomes. An introduction of genotype I into Brazil from Southeast Asia was confirmed in Bahia State, and full genome phylogeographic analysis revealed multiple introductions of DENV-4 genotype II in Brazil, providing evidence for at least 3 introductions of this genotype within the last decade: two from Venezuela to Roraima and one from Colombia to Amazonas. The phylogeographic analysis of full genome data has demonstrated the origins of DENV-4 associated with dengue outbreaks throughout Brazil.

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THE GLOBAL NGO DEWORMING INVENTORY: IDENTIFYING REPORTING GAPS EN ROUTE TO WHO GOALS FOR SOIL-TRANSMITTED HELMINTH CONTROL

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The World Health Organization (WHO)'s Preventive Chemotherapy (PCT) databank compiles annual data on treatments with mebendazole or albendazole for soil-transmitted helminthiasis (STH). These data are used to track progress towards WHO's goal of treating 75% of at-risk school-age children. National Ministries of Health (MoH) provide STH treatment reports to the PCT databank; the extent to which STH treatments by non-governmental organizations (NGOs) are included in these reports is unknown. To quantify this potential reporting gap, Children Without Worms (CWW) and WHO established the Global NGO Deworming Inventory. From June to December, 2011, CWW solicited data on STH treatments in 2010 from 120 NGOs. Repeated emails and telephone calls were made to encourage response. STH treatments reported by NGOs to the Inventory were compared with those reported by MoH to the PCT databank. Treatments with mebendazole or albendazole delivered as part of lymphatic filariasis or schistosomiasis control programs were excluded from analysis. Of 120 NGOs, 14 (12%) reported 65.4 million STH treatments of children aged 1-15 years, representing 25.1% of the 260.7 million treatments in the WHO PCT databank for 2010. Of these, 23.3 million treatments had not been reported previously to WHO by MoH and were therefore considered 'unique' to the Inventory. These 'unique' treatments, reported by 8 NGOs, accounted for 8.9% of all 2010 treatments in the PCT databank. Of these, 22.3 million (96%) were from 14 countries that had not submitted STH treatment reports to WHO. Limitations of the Inventory include low response rate, uncertainty about the 'universe' of all NGOs delivering STH treatments, and possible misclassification of Inventory-reported treatments as 'unique.' The Inventory identified reporting gaps between MoH and WHO, as well as between NGOs and MoH. To improve monitoring of STH treatments worldwide, reporting should be strengthened, focusing on the 8 NGOs and 14 countries identified by the Inventory.

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MODELING THE ECOLOGICAL NICHE OF HOOKWORM IN BRAZIL BASED ON CLIMATE

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The distribution of hookworm in schistosomiasis endemic areas in Brazil was mapped based on climate suitability. Known biological requirements of *Necator americanus* were fitted to data in a monthly long-term normal climate grid (18 km^2) using Geographic Information Systems (GIS) methods. Hookworm risk models were produced using the growing degree day (GDD) water budget (WB) concept. A moisture-adjusted model (MA-GDD) was developed based on accumulation of monthly temperatures above a base temperature of 15^{o}C (below which there is no lifecycle progression of *N. americanus*) conditional on concurrent monthly water budget values (rain/potential evapotranspiration) of over 0.4. A second model, designated the gradient index, was calculated based on the monthly accumulation of the product of GDD and monthly WB values (GDD x WB). Both parameters had a significant positive correlation to hookworm prevalence. In the arid northeastern part of the country, low hookworm prevalence was due to low soil moisture content, while the low prevalence in southern Brazil was related to low mean monthly temperatures. Both environmental temperature and soil moisture content were found to be important parameters for predicting the prevalence of *N. americanus*, the parasite favoring warm and moist thermal-hydrological regimes.

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SOIL-TRANSMITTED HELMINTH INFECTIONS AND PHYSICAL FITNESS OF SCHOOL-AGED BULANG CHILDREN IN RURAL SOUTHWEST YUNNAN, CHINA

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Chronic soil-transmitted helminth (STH) infections have tentatively been associated with reduced physical fitness. In order to assess the feasibility of measuring children's physical fitness under field conditions and to relate it to STH infections, we have conducted a cross-sectional survey among school-aged children of the Bulang ethnic group in rural southwest Yunnan, China. Standardized, quality-controlled methods were employed to determine STH infections (Kato-Katz technique), haemoglobin levels, anthropometry (body weight and height) and physical fitness (20-m shuttle run test). A compliance of 87% suggested good acceptance of the methods used. Among 69 children with complete data records, infection prevalence of *Trichuris trichiura*, *Ascaris lumbricoides* and hookworm were 81%, 44% and 6%, respectively. *T. trichiura* infection in children lowered the maximum volume of oxygen that can be utilized within 1 minute during exercise (VO_2 max estimate) by 1.94 ml kg^{-1} min^{-1} (P = 0.005) and resulted in 6.14 fewer laps (P = 0.004) completed as compared to children without *T. trichiura* infection. Additionally, the mean VO_2 max estimate of stunted children was lowered by 1.63 ml kg^{-1} min^{-1} (P = 0.002) and they completed 5.32 20-m laps less (P = 0.001) when compared to children of normal stature. No significant association between stunting and a current infection with any STH species could be established. As near-universal STH

infection is the rule among this population, stunting could result from previous chronic STH infection. In conclusion, implementation of physical fitness tests in rural, resource-constraint settings is feasible. To investigate our preliminary findings, we are currently conducting a double-blind, randomized, placebo-controlled trial to study the impact of de-worming on STH-infected children's physical fitness and strength. Measurements consist of parasitological examination (Kato-Katz and Baermann techniques) and determination of physical fitness (20-m shuttle run test) and strength (standing broad jump and grip strength test), anthropometry (body weight, height and thickness of skinfolds) and hemoglobin level. This set of parameters is being monitored over a 7-month period after treatment with triple-dose albendazole or placebo, and results will be available in time for the ASTMH meeting.

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INTESTINAL PARASITIC INFECTIONS AMONG PRIMARY SCHOOL CHILDREN IN NORTH JAKARTA: DOMINANCE OF SOIL TRANSMITTED HELMINTHS AND *BLASTOCYSTIS* INFECTIONS

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The prevalence of parasitic infection varies following the level of personal hygiene, sanitation and geography. Chronic/heavy infections in particular the Soil Transmitted Helminths (STH) affect the growth and development of a child, while in adults, reducing productivity and work capacity. The aim of this study was to determine the incidence of intestinal parasitic infections among school children in north Jakarta and identify the factors associated to high frequency of STH. A cross sectional study was conducted among primary school students. Stools were collected and examined by direct smear, formol ether concentration and culture for *Blastocystis hominis*. Standardized questionnaires was developed, consisted of demographic data, hand washing habit, nail hygiene, nail biting habit, play on the ground, food consumption from street sellers and defecation habit. A proportion of the students from grade 3-6 was randomly selected to receive the questionnaires. Observation on the students' nails were also done. Data was analyzed with SPSS 11.5 and any association between those variables with the frequency of STH infection was sought. There were 305 stools examined and 81.3% were positive for at least one species of intestinal parasites with 64.5% STH infection, 55.7% *B. hominis*, 15.1% *Giardia lamblia* and 3.1% *Entamoeba coli*. Intestinal helminths with highest frequency found was *Ascaris lumbricoides* (64.8%), followed by *Trichuris trichiura* (30.2%) and hookworm (3.0%). Intestinal helminths infection was not significantly associated with gender and age however there were significant asscociations the habits of hand contact with soil on the play ground, washing hand with soap after playing and the habit of cutting nail once a week ($p=0.018$, $p<0.05$). It is concluded that intestinal parasitic infections among the primary school children in North Jakarta was, high dominated by STH and *B. hominis*. Contact with soil was the main factor in this population to contract the STH infection. Hand washing with soap and regular cutting the nails were associated with lower infection rate. This attitude and risk should be communicate to and practised by the school children

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WORM THERAPY: HOW WOULD YOU LIKE YOUR MEDICINE?

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Worm therapy is attracting a lot of attention in the community of parasitologists. This form of therapy is undergoing clinical trial because many immunological diseases are considered to be poorly served by existing immune suppressive therapies, and because helminths have a seemingly innate ability to moderate the immune system. Clearly, however, parasitic worms have a dark side, and can be pathogenic. As a result, efforts are being made to harness the molecules produced by parasites, and responsible for moderating the immune system. This presentation will summarise : 1) the current state of play with regard to trials using the hookworm *Necator americanus,* and 2) the progress in the characterisation of the metabolomic profile of this parasite, as part of an effort to identify druggable immune suppressants.

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COST ASSESSMENT OF THE KATO-KATZ AND SPONTANEOUS SEDIMENTATION IN TUBE TECHNIQUE FOR THE DIAGNOSIS OF SOIL TRANSMITTED HELMINTH INFECTIONS IN DEVELOPING COUNTRIES

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Inexpensive, easy to carry out and highly sensitive diagnostic techniques are needed to estimate the real global burden of soil transmitted helminth (STH) infections in endemic populations. The Kato-Katz (K-K) technique is recommended as the gold standard for the diagnosis of STH by the WHO. The spontaneous sedimentation technique in tube (SSTT) is as sensitive as the K-K technique for the diagnosis of STH, but superior in detecting *Strongyloides* larvae. SSTT is easy to perform, simple to reproduce in field work conditions and theoretically inexpensive. The objective of this study was to compare the estimated costs to process and examine a single stool sample when using the SSTT and K-K techniques. Standard protocols of both techniques were performed during an epidemiological survey carried out in the Laboratory of Parasitology, Instituto de Medicina Tropical Alexander von Humboldt (IMTAvH), Lima, Peru. We considered an epidemiological scenario, where 100 samples are processed and examined per day, 5 days a week. Estimations (in US dollars) were based on the following parameters: laboratory materials, life expectancy of materials, time consumed in each process and salaries. The costs due to materials were $0.03 for both techniques. The average time needed to process and examine a single SSTT and K-K was 11 min 07 sec and 9 min 12 sec, respectively. This difference was dependant on the reading time being 3 min 06 sec for the SSTT and 1 min 21 sec for the K-K. The clearing time of the SSTT and K-K before the reading was 45 and 40 min, respectively. The estimated costs by salaries were $0.47 and $0.55 for a single K-K and SSTT, respectively. The total costs for a single K-K and SSTT were $0.5 and $0.58 respectively. We conclude that the SSTT is a cost-effective parasitological technique when compared to the K-K. Its costs differ slightly because more reading time was needed when using the SSTT to identify other parasite species such as protozoan and larvae. Our results should be considered in planning future epidemiological surveys and control programs in poor rural areas where STHs are still a public health problem.

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THE ROLE OF PUBLIC-PRIVATE PARTNERSHIPS IN INCREASING THE IMPACT OF NEGLECTED TROPICAL DISEASES CONTROL IN HAITIAN SCHOOLS

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The Haiti Neglected Tropical Diseases (NTD) Control Program currently distributes Albendazole and Diethylcarbamazine to combat two NTDs: Lymphatic Filariasis (LF) and Soil Transmitted Helminths (STH). This distribution is possible because of the collaborative efforts of a number of government and non-government partners (Ministries of Health and Education, USAID, RTI, Gates, CDC, IMA World Health, University of Notre Dame and others). In 2012, the program achieved national coverage, a tremendous achievement for a country that has had to overcome many challenges. While Mass Drug Administration (MDA) is effective and safe,

other preventative measures are also required to achieve the goal of eliminating these diseases. Children with bare feet are often most at risk for contracting hookworm, leading to severe anemia and malnutrition. Regular wearing of shoes has been proven to reduce the risk of hookworm transmission. IMA World Health (IMA) has partnered with TOMS Shoes to provide new shoes free of charge to Haitian children to help prevent STH. With every pair of shoes purchased, TOMS gives a new pair of shoes to a child in need through Giving Partners like IMA. In Haiti, the NTD Control Program's MDA distribution system was used to reach children with the greatest need for shoes. Partnerships were established between IMA, the Ministry of Education and NTD volunteers to facilitate the task of delivering shoes to school children immediately after MDA. School officials and volunteers ensured that children received shoes that fit correctly and that they actually wore the shoes they received. Between January 2011 to December 2011, hundreds of thousands of pairs of shoes were delivered in Haiti through IMA to help fight against NTDs. The long term goal for the program is to revisit the same children each year as they grow into new shoes. This successful example of collaboration between the private and public sectors has resulted in a more successful NTD control program in Haiti. Such partnerships can serve as a model that can be replicated in settings beyond Haiti for NTD control.

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MODELING THE POTENTIAL EPIDEMIOLOGICAL BENEFIT OF ADDING HOOKWORM VACCINE TO MASS DRUG ADMINISTRATION (MDA)

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While mass drug administration (MDA) for hookworm has successfully reduced morbidity among children under certain circumstances, its limitations (e.g., variable efficacy and often rapid post-treatment re-infection) have motivated the search for additional measures to reduce hookworm transmission, including a hookworm vaccine. A hookworm vaccine candidate is currently in development, however, the potential benefits of such a vaccine when incorporated into existing MDA programs has not been examined. We developed a dynamic compartment-based transmission model representing human and hookworm populations in a community to evaluate the potential impact of introducing a hookworm vaccine. Scenarios simulate the use of MDA (albendazole or mebendazole) alone and in conjunction with vaccination in higher and lower transmission settings. Use of vaccination in conjunction with MDA results in substantial decreases in community worm burden and egg excretion, yielding worm burden reductions of 71.9-96.0% for children, 49.1-78.9% for adults, and 52.9-83.3% for the overall population over 20 years of the intervention. These results are in comparison to decreases seen when MDA is used alone (74.9-94.0%, 25.1-46.1%, and 35.1-60.6% for children, adults, and overall population, respectively). Adding vaccination appears to interrupt transmission of hookworm infection by reducing the rate of increase in infection prevalence among children post-treatment while simultaneously reducing infection prevalence among adults. By interrupting transmission among both adults and children, vaccination in conjunction with MDA may lower infection prevalence substantially more than MDA alone.

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HUMAN INFECTIONS WITH *TRICHURIS TRICHIURA* ARE NOT ASSOCIATED WITH ALTERATIONS IN THE FAECAL MICROBIOTA: PRELIMINARY FINDINGS

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The human whipworm, *Trichuris trichiura,* is a highly prevalent and chronic helminth infection of children. *T. trichiura* colonises the large intestine where it may modify inflammatory responses either directly or indirectly through alterations in the intestinal microbiota. We hypothesised that human infections with *T. trichiura* would be associated with an altered faecal microbiota and that anthelmintic treatment would result in a microbiota that more closely resembles that observed in uninfected children.The study was done in two rural communities in Esmeraldas Province, Ecuador. School-age children were screened for the presence of *T. trichiura* infection in 3 sequential stool samples. Children were treated with albendazole (800 mg/day for 3 days) and ivermectin (single dose of 200 mg/kg), and stool samples were collected 3 weeks post-treatment. Stool samples were preserved in 70% ethanol at -20C and bacterial DNA was extracted using the FAST DNA Spin kit for soil. Bacterial community profiles were studied by 454 pyrosequencing of 16S rRNA genes. This generated ~470,000 sequences, which were processed and analysed using Mothur. Microbiota analyses were done using stool samples from 97 children aged 8-14 years of whom 17 were infected with *T. trichiura* alone, 48 were infected with *T. trichiura* and *Ascaris lumbricoides,* and 32 were not infected with any intestinal helminth. Post-treatment samples were analysed for 16 of the 17 children initially infected with *T. trichiura* alone and 21/32 uninfected children. Treatment resulted in 100% cure of intestinal helminth infections. Initial comparisons of the frequencies of OTUs representing specific bacterial phyla in paired samples before and after treatment from children with *T. trichiura* alone did not show significant differences. Preliminary analysis of the data does not support the hypothesis that *T. trichiura* infection alters the intestinal microbiota.

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DEVELOPMENT OF A RECOMBINANT ANTIGEN IMMUNOBLOT FOR THE DIAGNOSIS OF HUMAN BAYLISASCARIASIS

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Human baylisascariasis, caused by the raccoon roundworm *Baylisascaris procyonis*, is a disease that results from infection of a human host with *B. procyonis* L3 larvae, and the subsequent neural, visceral and ocular larva migrans syndromes that occur. *B. procyonis* has emerged in recent years as one of the most serious causes of larval migrans in humans. Due to the limited timeframe between onset of symptoms and severe mental deficits or death in heavy infections, early detection is imperative to minimize morbidity and mortality. Diagnostic assays using crude excretory-secretory antigen from *B. procyonis* infective larvae were useful, but cross-reactivity remained a problem. Toxocariasis is the most important parasitic infection needing to be serologically differentiated from *B. procyonis* because both parasites overlap with a similar epidemiology in temperate regions, and both infections show similar non-specific as well as clinical symptoms. Serological assays for baylisascariasis have recently been improved with the use of recombinant antigens and in this study, a Western blot assay

using a recombinant protein, rRAG1, was developed for the diagnosis of human baylisascariasis. The assay performance was assessed by testing 276 human serum samples: 17 from patients diagnosed with *Baylisascaris procyonis* infections, 109 from patients diagnosed with various diseases, and 150 from presumably healthy non-traveling US citizens. A sensitivity of 88.2% and a specificity of 97.3%, with no cross-reactivity with *Toxocara* positive serum, were observed with the rRAG1 Western blot.

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A FATAL CASE OF *HALICEPHALOBUS* FOUND ON AUTOPSY IN THE BRAIN OF A 65-YEAR-OLD FEMALE

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Halicephalobus is a saprophagous nematode that causes uncommon infections in man and other animals. Most frequently reported in horses worldwide, there have only been four previous human cases, all of which have been fatal. We report a fifth case, which occurred in a 65-year-old woman who presented with fever, nausea, and vomiting after being treated as an outpatient for a urinary tract infection approximately one month prior. During her course of stay at the hospital, the patient's consciousness gradually deteriorated and she had to be ventilated. Her mental status continued to decline and she was pronounced brain-dead. Prior to death, an MRI of her brain did not show any focal lesions. EEG showed moderate diffuse slowing for electrocerebral activity. Her past medical history included bipolar disorder, hypertension, osteoarthritis, and compression fractures. She was a smoker but denied alcohol or illegal substance abuse. Her only known animal contacts were her pet dogs and she had no recent documented international travel. Examination of brain tissue upon autopsy revealed numerous nematodes in the cortex, cerebellum, brain stem, and spinal cord morphologically consistent with *H. deletrix* (=*H. gingivalis*). Although this infection is extremely rare in humans, and risk factors are poorly understood, *Halicephalobus* should be part of the differential diagnosis, especially when *Strongyloides stercoralis*, which it resembles morphologically, is considered.

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POTENTIAL OF LIPID-CORE PEPTIDES AS AN EFFECTIVE DELIVERY SYSTEM FOR PARASITIC HELMINTH VACCINES

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Aspartic proteases initiate the proteolytic cascade of haemoglobin digestion within the gut of the blood-feeding helminths *Necator americanus* and *Schistosoma mansoni* and have been shown to be efficacious vaccine antigens in animal models of schistosomiasis japonica and hookworm infection, inducing inhibitory antibodies upon vaccination in the latter case. Particular epitopes of *N. americanus* hemoglobinase (*Na*-APR-1) are major targets of enzyme-neutralising antibodies and one of these, an exposed α-helical peptide (A_{291}Y), along with the equivalent region of *S. mansoni* cathepsin D (A_{262}K) have been synthesized as separate lipid-core peptide (LCP) constructs. LCPs are novel, synthetic, self-adjuvanting constructs consisting of one or more peptides linked to an adjuvanting lipid-core moiety. To assess the effectiveness of the LCP delivery system to induce antibodies against APR-1, LCPs containing the A_{291}Y or A_{262}K peptides (and GCN4, a yeast-derived peptide promoting α-helicity) were used to intraperitoneally immunise BALB/c and BR10. Br mice. The total IgG, IgG1 and IgG2a responses were measured by indirect ELISA. Antibody responses to both constructs were variable and dominated by IgG1 but the titres were considerably higher against the A_{262}K LCP. Induced IgG was purified and analysed for neutralising activity against recombinant *Na*-APR1. We demonstrated that anti-A_{291}Y-LCP IgG could inhibit the ability of recombinant *Na*-APR-1 to hydrolyse substrate by 78 – 100%. Interestingly, anti-A_{262}K-LCP IgG was also able to inhibit *Na*-APR-1 activity, albeit to a lesser extent. A *S. mansoni* vaccine trial is currently being conducted in mice using A_{262}K-LCP and results from this experiment will also be discussed. This is the first data presented using the LCP delivery system for helminth vaccines, which may overcome current vaccine development obstacles and prove a viable alternative to existing anti-helminth vaccine production and delivery.

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MALARIOMETRIC PARAMETERS EVOLUTION DURING THE CO-INFECTION *SCHISTOSOMA HEAMATOBIUM* AND *PLASMODIUM FALCIPARUM* IN MALI

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In sub-Saharan Africa, co-infection with multiple parasites is common. Schistosomiasis geographic distribution overlaps considerably with that of malaria in Mali. There is little understanding, however, of the biological interaction between *Schistosoma* and *Plasmodium* on human host. We investigated the potential for synergistic action by coinfecting pathogens on malaria indicators using an epidemiological framework in the endemic village of Dialakorodji. Between June 2005 and January 2006, we conducted a longitudinal cohort study with 316 children aged 11-14 years. At baseline, we estimated the prevalence of *S. haematobium* using urine filtration technique. Children infected with *S. haematobium* were matched to those without schistosomiasis according to sex, age, residence and use of preventive methods. Both groups of children were followed during malaria transmission which occurs from June to January. The prevalence of *S. haematobium* was 67.31%. Parasite rates were 12.97%, 20.73%, and 17.12% in June, October and January, respectively. Splenomegaly was observed 17.54%, 5.99% and 4.97% during these months. Gametocyte carriage was 0.31%, 3.66% and 2.20% in June, October, and January. Anemia prevalence varied from 4.11% (13/316) in June to 6.91% (15/217) in October. Anemia was higher in subjects co-infected ($p = 0.001$). The overall incidence of malaria was 8.23% (26/316). The average incidence of malaria was 13.33% in children infected with *S. haematobium* against 24.12% in those uninfected ($p = 0.15$). Parasite density was significantly higher among schistosomiasis free children in January compared to June or October ($p = 0.001$). Malaria incidence was significantly lower among children aged 11-14 years infected with S. *haematobium* compared with those *Schistosoma* uninfected ($p = 0.01$). Our results suggest a protective role in children infected by *S. haematobium* and *Plasmodium*.

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THE EUKARYOTIC INITIATION FACTOR 2 ALPHA KINASES: THE *PLASMODIUM* STAGE MANAGERS

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Regulation of mRNA translation plays a key role in controlling the life cycle of *Plasmodium* parasites, the causative agents of malaria. Sporozoites, the invasive form of malaria parasites transmitted by mosquitoes, are quiescent while in the insect salivary glands. Sporozoites only differentiate inside of the hepatocytes of the mammalian host. We show that sporozoite latency is an active process controlled by a eIF2α kinase (IK2) and a phosphatase. IK2 activity is dominant in salivary gland sporozoites, leading to an inhibition of translation and accumulation of stalled liver stage mRNAs into granules. When sporozoites are injected into the mammalian host, an eIF2α phosphatase removes the PO4 from eIF2α-P, and the repression of translation is alleviated to permit their transformation into liver stages. In *IK2* knockout sporozoites, eIF2α is not phosphorylated and the parasites transform prematurely into liver stages and lose their infectivity. The phosphorylation by another eIF2α kinase (PK4) of the regulatory serine 59 of *Plasmodium* eIF2α is essential

for the completion of the parasite's erythrocytic cycle that causes disease in humans. PK4 activity leads to the arrest of global protein synthesis in schizonts, where ontogeny of daughter merozoites takes place, and in gametocytes that infect *Anopheles* mosquitoes. Thus, to complete their life cycle, *Plasmodium* exploits the mechanism that regulates stress responses in eukaryotic cells.

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

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

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BACTERIAL CO-INFECTIONS IN NON-IMMUNE TRAVELERS WITH MALARIA: RATIONALE FOR ANTIBIOTIC THERAPY

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Management of malaria in non-immune travellers often include antibiotics in addition to antimalarial treatment, largely based on the risk of concurrent bacteraemia and high mortality observed in children with severe malaria in Sub-Saharan Africa. The importance of bacterial co-infections in patients diagnosed with malaria in non-endemic areas has however not been reported. A retrospective analysis of microbiology data was performed in 755 travellers diagnosed with malaria in Sweden. Bacterial cultures from blood and other locations were correlated to clinical outcome and antibiotic treatment. Blood cultures was drawn in 417 (53.8%) patients, and after excluding contaminants two bacterial isolates were of clinical relevance *Salmonella enteritidis* and *Escherichia coli*. Cultures from other locations, mainly urine and fecal samples, were taken in 63.2% patients of which 6.8% were positive. Antibiotics were administered in 28 (9.6%) of the patients, of which only five had positive bacterial cultures. C-reactive protein (CRP) levels at admission correlated with administration of antibiotics. In 75.0% of patients, the rational for antibiotic treatment was not confirmed by positive bacterial and/or radiological findings. Bacterial co-infection is uncommon among travellers diagnosed with malaria in Sweden. These data suggests a weaker association between malaria and bacteraemia than previously described in endemic settings and might indicate different pathophysiological mechanisms. Although antibiotics are crucial in patients with severe malaria and septicaemia, they might be given more often than empirically indicated.

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ASSOCIATION BETWEEN CYTOKINE AND TOLL-LIKE RECEPTOR GENE POLYMORPHISMS AND SEVERE MALARIA IN THREE REGIONS OF CAMEROON

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Plasmodium falciparum malaria remains one of the most widespread and deadliest infectious diseases in children under five-years. The disease has been a strong force for evolutionary selection in the human genome, and uncovering all of the human genetic factors that confer resistance would provide clues to the molecular basis of protective immunity that would be invaluable for vaccine development. To investigate malaria-associated polymorphisms in a Cameroonian setting, we recruited 892 children with cerebral malaria (CM) and/or severe malaria anaemia (SMA) or uncomplicated/mild malaria, plus 871 controls from 2 major ethnic groups in three regions with intense perennial *P. falciparum* transmission. Twenty nine polymorphisms in cytokine and toll-like receptor genes as well as the sickle cell trait were assayed on the Sequenom iPLEX platform. Our results reveal six and four SNPs associated with severe and mild malaria respectively. The AT, GT and AT heterozygous genotypes for the HbS (rs334), LTA (rs2239704) and IL-22 (rs1012365) genes conferred 73% (95%CI 0.14-0.52), 38% (95%CI 0.45-0.86) and 36% (95%CI 0.46-0.88) protection respectively against severe malaria anaemia whereas individuals with the TLR-9 (rs187084 CC), IRF-1 (rs2706384 AC/CC) and IL-17RD (rs6780995 GA) were more susceptible to cerebral malaria, hyperparasitaemia and hyperpyrexia respectively. Three SNPs were associated with protection from mild malaria in this study: GG, GA and GG genotypes of the IL-1A (rs17561), IL-17RE (rs708567) and IL-17RD (rs6780995) genes respectively were associated with 88% (95%CI 0.02-0.97), 37% (95%CI 0.45-0.88) and 50% (95%CI 0.31-0.80) protection while IL-13 (rs20541) was associated with increased risk to uncomplicated malaria and mild/moderate anaemia. Taken together, polymorphisms in human genes have important implication for susceptibility to paediatric malaria in Cameroon.

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THE RELATIONSHIP BETWEEN PARASITE GROUP A-LIKE VAR GENE EXPRESSION AND HOST MARKERS OF ENDOTHELIAL ACTIVATION

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Cytoadherence has been suggested to play a role in the pathogenesis of malaria. Cytoadherence is believed to be mediated via interaction of *Plasmodium falciparum* erythrocyte membrane protein-1 (PfEMP1) with a wide range of host receptors on endothelial cell and red blood cells. Var expression profiling of clinical *P. falciparum* isolates, based on PCR amplification of a conserved region within the DBLα domain of PfEMP1 and sequencing, revealed parasite predominantly expressing group A-like PfEMP1 types are associated with severe malaria. Moreover, we previously showed that expression of these group A-like PfEMP1 type is associated with the severe syndromes of impaired consciousness and severe malarial anemia, though not respiratory distress. Fatal *falciparum* malaria is accompanied by systemic endothelial activation and widespread induction of endothelial activation markers. It is believed that PfEMP1 mediated adhesion of parasitized red blood cell to the host microvasculature induces

endothelial activation compromising the vascular integrity. We explored whether endothelial activation markers such as angiopoietin-2 could explain the link between the expression of the group A-like PfEMP1 types and the clinical syndromes of severe malaria. To this end we measured angiopoietin-2 angiopoietin-1, sICAM1 and VEGF levels in the plasma of malaria patients from Kilifi, Kenya, presenting with either severe or non-severe malaria. We related these to the var expression profiles of the infecting parasites from each patient.

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MEASUREMENT OF BIOAVAILABLE IRON IN ERYTHROCYTES INFECTED WITH *PLASMODIUM FALCIPARUM* USING FLOW CYTOMETRY

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Iron is essential to both the human host and the malaria parasite. In the human host, iron homeostasis is regulated strictly at the level of intestinal absorption and by the innate immune response in response to infection. During its erythrocytic stage, *Plasmodium falciparum* requires iron for DNA synthesis, glycolysis, pyrimidine synthesis and electron transport. The host RBC contains 100fg (20mM) of iron; however, the majority of it is sequestered in heme which is incorporated into hemoglobin. While it is unclear what the parasite uses as its source of iron, most evidence indicates that the parasite does not obtain iron from hemoglobin, but from another an intra-erythrocytic bioavailable pool of iron. We developed flow cytometry based method for determining the bioavailable iron content of parasitized erythrocytes using the nucleic acid dye Syto61 and the iron sensitive dye Calcein AM. This new approach has allowed us to systematically study changes in bioavailable iron in parasitized cells through the course of the erythrocytic life cycle and in response to the addition of host serum iron sources and iron chelators. We found that bioavailable iron content increases with the development of the malaria parasite from early ring to the trophozoite stage, and that the addition of either transferrin or ferric citrate to culture media increases the bioavailable iron found in late stage trophozoites. Additionally, this method has allowed us to examine the impact of iron chelators with known anti-malarial activity on the bioavailable iron of parasitized RBCs. Our novel method for detecting bioavailable iron within malaria parasitized RBCs provides an important tool for elucidating the mechanisms by which the malaria parasite senses, acquires, stores, and regulates iron during the erythrocytic stage of its life cycle.

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MALARIA AND PRE-ECLAMPSIA IN AN AREA WITH UNSTABLE MALARIA TRANSMISSION IN CENTRAL SUDAN

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Placental malaria and pre-eclampsia occur frequently in women in tropics and are leading causes of maternal and perinatal morbidities and mortality. Few data exist concerning the interaction between placental malaria and pre-eclampsia. A case control study was conducted in Medani Hospital, which locates in an area of unstable malaria transmission in Central Sudan. Case (N= 143) were women with pre-eclampsia, which was defined as systolic blood presure≥140 mm Hg or diastolic blood pressure ≥ 90 mm Hg and proteinuria. Controls were parturient women (N =143) without any blood pressure values >139/89 mm Hg or proteinuria. Obstetrical and medical characteristics were gathered from both groups through structured questionnaires. Placental histopathology examinations for malaria were performed. Twenty-eight (19.6%) vs. 16 (11.2%); P = 0.04 of the cases vs. controls, had placental malaria infections. Five (2%), 1 (2%) and 22 (28.0%) vs. 1, 2 and 13 of the placentae showed acute, chronic and past infection on histopathology examination in the two groups respectively, while 115 (80.4%) vs.127 (88.8%) of them showed no infection, P = 0.04. In multivariate analysis, while there were no associations between age, parity, educational level, lack of antenatal care, blood groups and body mass index and pre-eclampsia; family history of hypertension and placental malaria (OR =2.3, 95% CI=1.0-5.2; P = 0.04) were significantly associated with pre-eclampsia. In conclusion, Placental malaria was associated with pre-eclampsia. Further research is needed.

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NEAR-INFRARED FLUORESCENT IMAGING: A NOVEL TECHNIQUE TO ASSESS INFLAMMATION IN EXPERIMENTAL CEREBRAL MALARIA

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Cerebral malaria (CM) is the deadliest complication from *Plasmodium falciparum* infection, and its pathophysiology is poorly understood. Metalloproteinases (MMPs) are proteolytic enzymes responsible for both the breakdown of the extracellular matrix and upregulation of inflammation, and are reportedly increased in infections with protozoan parasites, including *P. falciparum*. We assessed the activity of MMPs in our murine model of cerebral malaria via near-infrared (nearIR) fluorescent imaging using a probe which emits nearIR fluorescence once broken down by MMPs. C57Bl/6 mice infected with *P. berghei* ANKA (PbA), a murine plasmodium species which causes experimental cerebral malaria (ECM), were compared to mice infected with *P. berghei* NK65 (a strain which does not result in ECM in this mouse model) and to uninfected control mice. We observed significantly higher inflammation in the liver and spleen of both strains of infected mice compared to control. However, there was a significant effect of PbA infection on mean expression of MMP in the brain when compared to the other groups, indicating that the degree of brain inflammation was specific to ECM and to cerebral damage during infection with PbA. Some brain regions such as the cerebellum, the hypothalamus and the cortex exhibited earlier changes in MMP expression compared to other areas of the brain. Over time, there were significant increases in the degree of brain inflammation in PbA infected mice, corroborating the tropism of this strain to cerebral damage and confirming that higher level of inflammation are associated with the severity of disease in ECM. This imaging technique provided sensitive and readily applicable method of monitoring disease and may prove valuable in the evaluation of response to therapy during cerebral malaria and may be employed to create a platform capable of analyzing inflammatory changes in the course of disease.

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MALARIA AND CANCER: THE IARC MONOGRAPHS EVALUATION AND RATIONALE

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In February 2012, 26 scientists from 11 countries met at the International Agency for Research on Cancer (IARC/WHO) in Lyon, France to evaluate the carcinogenicity of malaria. These assessments will be published in Volume 104 of the IARC Monographs. In 1962, Denis Burkitt first noted a very strong geographical association between holoendemic malaria and the most frequent paediatric cancer in sub-Saharan Africa, endemic Burkitt lymphoma (eBL). Epstein-Barr virus (EBV), a ubiquitous oncogenic herpesvirus is recognized as a necessary agent for the pathogenesis of eBL. Its persistence in over 90% of adults is usually benign and generally only causes disease when the balance between the virus and host immune system is upset. African children are infected by EBV early in life (< 3 years of age) and the timing of EBV and *Plasmodium* co-infection and the intensity of malarial infections at an individual level seem to influence the outcome of EBV dysregulation, which may lead to eBL. Multiple correlation studies have strongly linked the incidence of eBL to areas where *P.*

falciparum transmission is holoendemic. In two case-control studies among children living in holoendemic malaria areas, positive associations were observed between eBL and high titres of total IgG antibodies specific to whole schizont extracts; this risk was increased significantly in conjunction with high EBV antibody titers. Different mechanisms may explain the joint contribution of EBV and chronic *P. falciparum* infection to eBL. *In vitro* and *in vivo* data indeed demonstrate that *P. falciparum* can disturb the immature immune system in young children by expanding the B-cell pool in which eBL arises, and reactivates EBV known to be causally associated with this tumour. By evaluating the evidence in humans for the carcinogenicity of holoendemic malaria judged to be "*limited*" together with the strong mechanistic evidence, the Working Group concluded that malaria caused by infection with *Plasmodium falciparum* in holoendemic areas is *"probably carcinogenic to humans" (Group 2A).*

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PERSISTENCE OF *PLASMODIUM FALCIPARUM* HISTIDINE-RICH PROTEIN 2: MAY THE PREVIOUSLY INFECTED RED BLOOD CELLS STAND UP

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Biomarkers of malaria such as *Plasmodium falciparum* Histidine-Rich Protein 2 (PfHRP-2) are increasingly being used for routine diagnosis of *falciparum* malaria and as a measure of total parasite burden. PfHRP-2 persists in circulation for up to 4 weeks even after successful treatment raising questions of why and where in the blood does this biomarker persist. To answer the question of where, PfHRP2 concentration in blood compartments (red blood cells and plasma) of volunteers taking part in an anti-malarial clinical trial (KEMRI SSC #s 1420) was measured by ELISA on day of enrollment, following treatment, and thereafter on days 1-7, 9, 14, 21 and 28. Malaria parasitemia was also determined by microscopy, plasmodia LDH and PCR. We were surprised to find that, after successful treatment, PfHRP2 persisted up to 28 days, not in the plasma, but in the RBCs. Absence of malaria parasites for the period of the study was confirmed by microscopy, pLDH or PCR. The data presented here dispel the assumption that the long half-life of PfHRP-2 in blood represent slow plasma clearance rate. The persistence of PfHRP-2 in RBCs in absence of parasitemia can be explained by three scenarios: 1) that PfHRP2 survives in RBCs containing dead malaria parasites and such RBCs are cleared gradually. 2) That PfHRP2 survives in previously infected RBCs that have been allowed back in the peripheral circulation after the malaria parasites have been removed by the "pitting" action at the reticuloendothelial organs. 3) By binding to normal uninfected RBCs through receptors such as CR1.

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OVER EXPRESSION OF HISTONE DEACETYLASE 1 PROTEIN IN *PLASMODIUM FALCIPARUM* RESULTS IN DOWN-REGULATION OF ENDOGENOUS PROTEIN EXPRESSION

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Histone deacetylases (HDACs) are enzymes that, together with other regulatory proteins, reversibly modify lysine residues on the N-terminal tails of histones, thereby contributing to regulation of chromatin-structure and gene expression. HDACs are validated therapeutic targets for cancer and other diseases, and are a potential new antimalarial drug target. There are five HDAC homologues present in the *Plasmodium falciparum* genome, including PfHDAC1 which has been shown to be a target of antimalarial HDAC inhibitors such as SAHA. Recent data has shown that HDAC inhibitors hyperacetylate *P. falciparum* histone and non-histone proteins, and cause genome wide transcriptional alterations. Two HDAC inhibitors have also been shown to inhibit the exo-erythrocytic growth of *P. berghei* in HepG2 liver cells. Together these data underscore the potentially important roles for *P. falciparum* HDACs on parasite growth and development and the need to characterize the biology of these enzymes in different parts of the *Plasmodium* life cycle. To begin to address this, we transgenically over-expressed PfHDAC1 with a C-terminal c-myc epitope tag in situ in *P. falciparum* parasites. Our data confirmed that the PfHDAC1cmyc protein is expressed in the transgenic parasite line, but that as a result endogenous PfHDAC1 expression is reduced. The resulting phenotype of the over-expression line was unchanged compared to wild type non-transfected parasites. There was no change in the intraerythrocytic developmental cycle, levels of total deacetylase activity, or sensitivity to different HDAC inhibitors. Our findings indicate that PfHDAC1 expression is tightly regulated, providing further evidence that this enzyme is a promising new drug target in *P. falciparum*.

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REVERSAL EFFECT OF POTENT ANTIPLASMODIAL ANNONACEOUS PLANT EXTRACTS AGAINST CHLOROQUINE RESISTANT *PLASMODIUM FALCIPARUM*

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Widespread resistance to old antimalarials and emergent resistance to artemisinin-based drugs highlight the urgent need for alternative therapies. In recent years, the focus has been on identifying effective reversers of chloroquine resistance. One of the promising approaches to achieve this might be to combine previously effective antimalarials with potent antiplasmodial plant extracts. In this study, we have evaluated the potential of antiplasmodial extracts from three Annonaceous plants to reverse the *P. falciparum* resistance to chloroquine. Thirty two *P. falciparum* isolates were collected from malaria patients and cultured *in vitro* in the presence of combined drugs. The genetic diversity of isolates was determined according to their msp1, msp2 and glurp alleles. The resistance markers to chloroquine (Pfcrt K76T) and sulfadoxine (dhps A437G) were also determined by RFLP. The phylogeny tree of isolates was built, base on the type of interaction observed and the presence of resistance markers. Results showed that antiplasmodial activity was more significant with combinations, with an average X50 potency magnification. In addition, 11 isolates were sensitive to chloroquine and sulfadoxine, 4 were resistant to both drugs, 8 were resistant to choroquine and sensitive to sulfadoxine, and 9 were sensitive to chloroquine and resistant to sulfadoxine. The interactions were mostly additive with few cases of synergism and antagonism. The interaction between combined drugs is likely related to intrinsic characteristics of each isolate, which could be also related to the level of immunity of the human host. These findings highlight the promise of *P. falciparum* chloroquine resistance reversers discovery, and support the further study of the investigated plant extracts for improve candidates for chloroquine-natural products combinations.

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PLASMODIUM FALCIPARUM CARBONIC ANHYDRASE - A POTENTIAL NEW ANTIMALARIAL DRUG TARGET

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Malaria is a significant global infectious disease, resulting in ~1 million deaths annually. There is currently no vaccine and many of the available drugs are becoming less effective due to parasite resistance. This means that we need to identify new drugs, preferably those with novel modes of action, to help prevent issues of cross-resistance. Carbonic anhydrase

inhibitors have been used clinically for over fifty years and have recently been identified as a possible new antimalarial drug class. In this study a panel of clinically used carbonic anhydrase inhibitors containing primary sulphonamides was first screened for *in vitro* activity against *Plasmodium falciparum* 3D7 parasites. Poor activity (IC_{50} >12 μM) was observed for these compounds. In contrast, when the antimalarial activity of a panel of novel synthetic primary sulfonamide compounds was tested, five compounds were found to have IC_{50s} between 0.7 - 4 μM, and good selectivity for *P. falciparum* versus mammalian cells (SI = 25-85). Analogues of these compounds lacking the primary sulfonamide group were then synthesised to allow the impact of the primary sulfonamide functional group on antimalarial activity to be evaluated. These analogues displayed decreased activity against *P. falciparum,* providing evidence that the primary sulfonamide may be responsible for the antimalarial activity of these compounds. This study has identified novel primary sulfonamide compounds as hits for development of new antimalarial drug leads. These compounds may allow the investigation of *P. falciparum* carbonic anhydrase as a potential new antimalarial drug target.

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BIOACTIVITY-GUIDED ISOLATION AND IDENTIFICATION OF ANTIPLASMODIAL CONSTITUENTS OF *STACHYTARPHETA CAYENNENSIS* LEAF EXTRACT

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Malaria causes a large number of infections and deaths annually, with a negative impact on economic development in affected countries. Attempts to develop a malaria vaccine that confers immunity against *Plasmodium* have been hampered by the complexity of their multistage development, added to their impressive antigenic variability. Drug treatment remains the mainstay for malaria control but there are limited drugs available and the emergence of drug resistance also poses a threat to the effective treatment of malaria. Hence, the search for new drugs remains crucial. The indigenous use of plants as medicine has been exploited for the discovery of new drug molecules. *Stachytarpheta cayennensis* is used in Nigeria and many tropical countries as a remedy for symptoms of malaria. This study investigates the presence of antiplasmodial constituents of *S. cayennensis* with potential for development as malaria medicine, through activity - guided separation. Dried powdered leaves of *S. cayennensis* were successively extracted using n-hexane, dichloromethane, methanol and water. Successive extracts were subjected to 4-day test in mice against *P. berghei*. The methanol extract was most effective, significantly ($P<0.05$) suppressing infection by 96.55 % at a dose of 100 mg/kg. This extract was partitioned into ethylacetate and water portions and then the ethylacetate portion was further separated into 11 sub fractions (SCA1 - SCA11) by column chromatography using a gradient mobile phase system of hexane, ethylacetate and methanol. The sub fractions and water portion were tested against chloroquine sensitive (HB3) and chloroquine resistant (FCM29) *P. falciparum*. Results showed that SCA10 was most active, with IC_{50} values of 9.2 and 10.89 μg/mL against *P. falciparum* HB3 and FCM29 respectively. Chromatographic analysis of SCA10 showed the presence of a sterol glycoside, using sitosteryl glycoside as a marker. This suggests that the ethylacetate sub fraction and the identified compound account for the antiplasmodial effects of *S. cayennensis* and show potential for development as antimalaria medicine.

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ANTIMALARIAL ACTIVITY OF A NOVEL HDAC INHIBITOR; SB939

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Antimalarial drug resistance is an increasing problem around the world and is driving the need for new antimalarial agents that act on novel parasite targets. Histone deacetylase (HDAC) enzymes, which are being targeted for cancer and other diseases, are also potential new drug targets in malaria parasites. A number of HDAC inhibitors have already shown potent and selective *in vitro* activity against *Plasmodium falciparum* parasites; however these compounds generally have poor pharmacokinetic profiles that impact on *in vivo* efficacy. In order to overcome this problem, new HDAC inhibitors are being developed, including SB939 (S*BIO, Singapore), a potent hydroxamate-based HDAC inhibitor currently in clinical trials for treatment of cancer. SB939 has a better pharmacokinetic profile than first generation HDAC inhibitors such as suberoylanilide hydroxamic acid (SAHA), a clinically approved anti-cancer compound. Our studies show that SB939 and SAHA have similar *in vitro* potency against *P. falciparum* asexaul stage parasites (IC_{50} ~100-200 nM) and against exo-erythrocytic stage *P. berghei* parasites growing in HepG2 liver cells (IC_{50} ~150 nM). Orally administered SB939 (25mg/kg; BID for 3 days) was found to significantly inhibit *P. berghei* ANKA parasite growth *in vivo*, preventing development of cerebral malaria-like symptoms in an experimental cerebral mouse malaria model. These results underscore the potential of HDAC inhibitors as a promising new antimalarial drug class.

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ANTIPLASMODIAL ACTIVITY OF CHLOROQUINE ANALOGS METALLODRUGS AND OF OTHER METAL COMPLEXES

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One of the recent strategies to search new antimalarials is to develop metal complexes from existing old antimalarials. Metallodrugs are used as first line treatment against cancer and parasitic diseases. In this study, several potential metallodrugs were synthesized and evaluated for biological activities: (i) aryl hydrazones (AHR), with known pharmacological applications (antimicrobial, anticonvulsant, analgesic, anti-inflammatory and anticancer) complexed with gallium (Ga); (ii) new ferrocene-derived molecules, a promising class of antimalarials undergoing clinical screening, e.g. ferroquine; (iii) chloroquine analogues (monoquinoline, MAQ, and, bisquinoline, BAQ) complexed with platinum, palladium and iron. The compounds were tested for activity against *Plasmodium falciparum* chloroquine resistant, W2 clone, and for cytotoxicity using a hepatoma cell line. The selectivity index (SI), a ratio between toxicity and activity showed that: three AHR had SI up to 5314; after gallium complexation they became toxic, with a lower SI (10 to 600); six ferrocene-tetrasubstituted olefins were all toxic (SI <10); MAQ and BAQ, tested after complexation with Fe, Pt or Pd were more active than the original compounds (higher SI, 98 to 4405). The chloroquine analogues significantly inhibit hemozoin formation *in vitro*. MAQ was more active than BAQ and CQ (the doses inhibiting hemozoin formation were, 0.62, 2.5 and 2.5mg/mL, respectively), as further confirmed by docking studies. MAQ Fe, MAQ Pt, BAQ Fe and BAQ Pt inhibited the formation of hemozoina from 0.62mg/mL, MAQ Pd was was less active (5mg/mL). The both aminoquinoline derivatives interacted with dimeric hematin to form a complex, like CQ; they were weak PfLDH inhibitors In conclusion, the metal complexation enhanced the activity of chloroquine-like drugs, although MAQ had a higher selectivity index than BAQ, it was as active as CQ. The active

metallodrugs will be assayed ex-vivo against *P. vivax* and *P. falciparum* human fresh isolates to clarify whether they overcome chloroquine-resistance.

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ANTIMALARIAL EFFECT OF ANTHRAQUINONE, NITRIC OXIDE DONOR (SODIUM NITROPRUSSIDE) ITS INHIBITOR (L-NAME) AND THEIR COMBINATIONS ON *PLASMODIUM BERGHEI*

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Combination therapy is presently adopted in malaria treatment.Nitric oxide (NO) is reported to aid the chemotherapy of parasitic diseases, while anthraquinones(ANT) are known to induce NO production. The combination of anthraquinone,NO donor and inhibitors in the treatment of malaria have not being investigated.This study examined the curative effect of ANTalone and in combination with NO donor (sodium nitroprusside) and inhibitor N-NitroL-arginine methyl ester (L-NAME) on malaria parasite with a view of improving the efficacy of the combination. *Plasmodium berghei* (NK65 Chloroquine- sensitive strain) was inoculated intraperitoneally (i.p) into fifteen groups of mice containing 5-6 swiss albino mice per group and were left for 240 hr (10 days) before commencement of treatment. 25, 50 and 100 of ANT, CQ (10 mg/kg),0.5 (SNP) and 40 mg/kg L-NAME and their combinations were administered to the mice by the oral route from day 12 (day 12) of infection up to day 15. Average parasitaemia were determinated. Daily body temperature measured with a digital thermometer.The estimation of nitric oxide level in the plasma of mice was adopted.Values were expressed as mean ± S.E.M. The results obtained were compared using ANOVA, followed by Student-Newman- Keul's test. The results show that parasitemia in infected mice was significantly decreased at 15 days after infection from 27.0% to between 7.4 -14.6% when 25-100 of ANT were administered, 0.5 mg/kg SNP gave 8.56%, 40 mg/kg L-NAME exhibited 4.6% while 10 mg/kg CQ showed 0.06% parasitaemia. With the combination therapy, SNP + ANT gave 7.98 - 19.3%, L-NAME + ANT showed 9.25 - 11.39%, CQ + SNP and CQ + L-NAME exhibited 0.45 and 0.08 % parasitaemia respectively.% chemo-suppression observed with ANT 50mg/kg was the highest for the ANT alone, being 72.5%, while those obtained for ANT 25 and 100mg/kg were 0% and45.7% respectively. SNP alone gave 68.2% and its combination with ANT gave a chemo-suppression of 44.9,28.3and70.3% respectively. L-NAME alone gave 82.9 while the combination of L-NAME and ANT 25-100mg/kg gave 65.6, 77.4 and 57.7% respectively. CQ alone, CQ in combination with SNP and L-NAME showed the highest % chemo-suppression of 99.7, 98.3 and 99.7% respectively. In conclusion, ANT possesses some antimalarial activity. SNP did not significantly improve the curative activity of ANT. Antimalarial activity of ANT could be explained through the NO generated.

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A RANDOMIZED, PLACEBO-CONTROLLED STUDY TO EVALUATE THE EFFECT OF TAFENOQUINE ON THE ELECTROCARDIOGRAM (ECG), WITH FOCUS ON CARDIAC REPOLARIZATION (QTC DURATION) IN HEALTHY SUBJECTS

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Tafenoquine (TQ) is an 8-aminoquinoline in co-development by GlaxoSmithKline and the Medicines for Malaria Venture for the radical cure of *Plasmodium vivax* malaria as a single dose in combination with chloroquine. Due to the long half life of TQ (2-3 weeks) we performed a parallel, single blind thorough QT (TQT) study. The primary objective was to demonstrate a lack of effect of TQ on QTcF following a supra-therapeutic dose compared to placebo. Secondary objectives included the effect of lower doses of TQ on QTcF and related ECG parameters, PK/PD relationships and TQ safety and tolerability. TQ was dosed matching the ongoing phase IIb dose ranging study with an added, FDA required, supra-therapeutic arm. 260 non-glucose-6-phosphate dehydrogenase deficient (quantitative phenotypic assay) healthy volunteers fulfilling entry criteria were randomized (n=52 per arm) to receive either placebo, moxifloxacin 400 mg (positive control) or TQ (300, 600 or 1200 mg). On days 1 and 2 all subjects received placebo except those in the supra-therapeutic arm who received three daily doses of 400 mg. Parameters monitored included adverse events, vital signs, laboratory tests, TQ PK and both Holter and paper ECG recordings. Study drugs were well tolerated. The primary endpoint was change from baseline in QTcF for the supra-maximal arm compared to placebo. A lack of effect will be demonstrated (or null hypothesis will be rejected) when the upper 90% confidence interval for the difference in change from baseline for all time points is less than 10 msec using a repeated measures analysis of covariance (ANCOVA), fitting subject as random effects and time, treatment, time by treatment interaction as fixed effects with baseline QTcF as a covariate. Secondary endpoints include PK parameters AUC(0-t), C_{max}, and t_{max} of TQ derived from plasma concentrations. We will present relevant data in full including appropriate PK/PD modeling. Conclusions regarding TQ's potential for elongation of the QT and implications for future use of the drug will then be discussed.

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A GENOMICS PLATFORM FOR ANTI-MALARIAL DRUG DISCOVERY

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Genomics technologies are greatly enhancing our understanding of the basic biology of the malaria parasite. However, malaria remains a global challenge, causing more than 250 million infections and 1 million deaths annually. The rapid emergence of drug resistance threatens eradication efforts. Understanding drug mechanism of action (MOA) and the molecular basis of multi-drug resistance (MDR), as well as predicting effective and combinatorial drug interactions from genomics data, could be dramatically enhanced by tapping into the rich genomic information. To test the utility of gene expression responses in predicting drug MOA, we developed a predictive framework that harnesses genome-wide transcriptional responses to drugs targeting a diverse array of biological pathways. A high density, multi-sample gene chip developed in our lab was applied in the identification of specific gene expression signatures that are predictive of drug MOA. In particular, two strains of the malaria parasite were briefly exposed to 10 different drugs targeting folate biosynthesis, heme detoxification, DNA repair, mitochondrial protein synthesis and electron transport. We developed a simple heuristic that makes no assumptions about the up- or down-regulation of specific genes by a given drug but instead leverages a genome-wide signature. This method was applied to the gene expression responses of the 2 laboratory strains and correctly predicted the expected MOA of six out of nine drugs ($P = 0.0002$) whose primary MOA was known. The gene expression signatures of drugs with the same MOA were found to be more similar to each other compared to that of drugs that have different MOA. In addition, we developed computational methods for the de novo prediction of MOA and regulatory effectors driving the drug induced transcriptional changes. This approach will be extended to other compounds targeting diverse pathways in the malaria parasite to create a reference resource and computational tools for robust prediction of drug MOA.

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IDENTIFICATION OF NOVEL ANTI-MALARIAL CHEMOTYPES THROUGH A SYSTEMATIC SCREENING OF KINASE INHIBITOR LIBRARY

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Malaria is a devastating disease transmitted by infected mosquitoes, affecting half the world's population. Due to increasing drug resistance and high cost of current therapy, a need for a new class of anti-malarial drugs exists. We identified lead chemotypes with *in vitro* activity against *Plasmodium falciparum* through a systematic screening of 34 diverse structural classes from a large library of kinase inhibitors. The initial screening plate, comprised of 88 compounds, was screened for *in vitro* activity against erythrocytic stages of *P. falciparum* using a standard ^{3}H-hypoxanthine incorporation assay at a single concentration (2.5 or 2 μM), with follow-up IC_{50} determinations on active compounds. Compound series were selected based on activity and after three additional rounds of screening, five structurally distinct compound series were identified with *P. falciparum* IC_{50} values ranging from 100 nM to 2 μM. Three of the five compound series have been prioritized for further investigation, including assessment of ADME properties and additional activity assays (parasite reduction ratio and assessment on chloroquine resistant strain K1). Exploration of SAR addressing the activity profile and drug-like properties is ongoing. In conclusion, we have identified three potential lead series that show promising activity with a clear path forward for potential improvements.

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PROVEBLUE (METHYLENE BLUE) AS AN ANTIMALARIAL AGENT

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Proveblue (international patent no PCT/FR/2007/001193), which is a methylene blue preparation that complies with the European Pharmacopoeia and contains limited organic impurities and heavy metals of recognized toxicity, demonstrated to possess *in vitro* antimalarial activity (at a geometric mean IC_{50}s of 3.62 nM) against 23 *Plasmodium falciparum* strains that are resistant to various other antimalarials. No significant association was found between Proveblue IC_{50} and polymorphisms in the genes that are involved in quinoline resistance, such as pfcrt, pfmdr1, pfmdr2, pfmrp and pfnhe-1; furthermore, there was no significant association between Proveblue IC_{50} and the copy numbers of pfmdr1 and pfmdr2. While Proveblue was shown to have antagonistic effects in combination with chloroquine and additive effects in combination with monodesethyamodiaquine against the nine *P. falciparum* strains, Proveblue presented exhibited noticeable synergistic effects in combination with mefloquine and quinine and high synergistic effects in combination with dihydroartemisinin. In addition, we demonstrated that there was no significant correlation between dihydroartemisinin and Proveblue IC50 ($r2 = 0.056$; $P = 0.275$). All of these data suggest that Proveblue could be effective as a good partner with artemisinin derivatives. These results confirm the therapeutic potential of Proveblue, which could be integrated into new, low-cost, antimalarial combination therapies.

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NETWORK BIOLOGY: A TOOL FOR UNDERSTANDING DRUG MECHANISM OF ACTION IN THE MALARIA PARASITE

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Understanding the mechanism of action of drugs is an important yet one of the least understood steps in the discovery of new drugs for the eradication of infectious disease. Here we demonstrate the utility of combining gene expression profiling and quantitative genetics with powerful computational tools to uncover the mechanism of action and a strain dependent effect of a DNA damaging agent in the malaria parasite. Based on gene expression networks constructed from chloroquine resistant (CQR) and sensitive (CQS) malaria parasites, we show how divergence in gene interactions between the chloroquine resistance transporter (pfcrt) and msh6, a gene involved in DNA damage repair predicts sensitivity to the drug methyl methane sulfonate (MMS). Quantitative trait loci (QTL) mapping of the dose response to this drug highlights the genetic locus encoding the msh6 gene, confirming that differential wiring of genes can influence drug response in a predictable manner. In addition, QTL mapping reveals that the sensitivity to MMS is dependent on a genetic interaction, epistasis, with an additional locus that includes an AP2 transcription factor (PFD0985w) whose interaction with pfcrt diverges between CQR and CQS parasites. Using computational methods for reverse engineering transcription factor targets from gene expression data, we find that genes co-regulated with this AP2 are enriched for DNA damage repair functions. By combining QTL analysis and network biology in a novel way we can begin to dissect the complexity of understanding drug mechanism of action.

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L-ARGININE INFUSION IN SEVERE *FALCIPARUM* MALARIA: A PILOT STUDY OF SAFETY, PHARMACOKINETICS AND EFFICACY

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Decreased nitric oxide (NO) and hypoargininemia are associated with severe *falciparum* malaria and may contribute to severe disease. Intravenous L-arginine increases endothelial NO in moderately-severe malaria (MSM) without adverse effects. Safety and efficacy of L-arginine in severe malaria have not been assessed. In an open-label pilot study in adults with severe malaria, patients were randomized to L-arginine infusion over 8 hours compared to saline. All patients received intravenous artesunate. Vital signs, selected biochemical measures (including blood lactate and plasma L-arginine) and endothelial NO bioavailability (using reactive hyperemia peripheral arterial tonometry (RH-PAT) were assessed serially. Pharmacokinetic analyses of L-arginine levels were performed using NONMEM. Six patients received L-arginine and two received saline and there were no deaths in either group. There were no changes in mean systolic (SBP) and diastolic blood pressure (DBP) or other vital signs with L-arginine, but a transient but clinically unimportant mean maximal decrease in SBP of 14 mmHg was noted. Although no changes in mean potassium, glucose, bicarbonate, pH or phosphate were seen, there were transient but clinically insignificant mean maximal increases in potassium of 0.3mmol/L, and mean maximal decreases in glucose of 0.8mmol/L and bicarbonate of 2.3mEq/L with L-arginine. There was no effect on lactate clearance or RH-PAT index. Pharmacokinetic modelling (n=4) showed L-arginine concentrations 40% lower than predicted from a model developed in MSM. We found L-arginine infused at 12g over 8

hours in severe malaria was safe, but had no effect on lactate clearance or endothelial NO bioavailability. Future studies may require increased doses of L-arginine.

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A ROADMAP TOWARDS THE DEPLOYMENT OF PRIMAQUINE IN AFRICA TO REDUCE TRANSMISSION OF *PLASMODIUM FALCIPARUM*

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Following the recent successes of malaria control in sub-Saharan Africa, the gametocytocidal drug primaquine needs evaluation as a tool to further reduce the transmission of *Plasmodium falciparum* malaria. The drug has scarcely been used in Africa because of concerns about its safety in people with glucose-6-phosphate dehydrogenase (G6PD) deficiency. The evidence base for the use of primaquine as a transmission-blocker is limited by a lack of comparable clinical and parasitological endpoints between trials. In March 2012, a group of experts met in London to discuss the existing evidence on the ability of primaquine to block malaria transmission, to define the roadblocks to the use of primaquine in Africa and to develop a roadmap to enable its rapid, safe and effective deployment. We present the outputs of this meeting; a strategic plan to optimize trial design to reach desired goals efficiently. The roadmap includes suggestions for a series of phase 1, 2, 3 and 4 studies to address specific hurdles to primaquine's deployment. These include ex-vivo studies on efficacy, primaquine pharmacokinetics and pharmacodynamics and dose escalation studies for safety in high-risk groups. Phase 3 community trials are proposed, along with Phase 4 studies to evaluate safety, particularly in pregnancy, through pharmacovigilance in areas where primaquine is already deployed. In parallel, efforts need to be made to address issues in drug supply and regulation, to map G6PD deficiency and to support the evaluation of alternative gametocytocidal compounds.

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BACTERIA DIVERSITY IN THE MIDGUT OF WILD MOSQUITO VECTOR *ANOPHELES GAMBIAE*: A STEP TOWARD FINDING SUITABLE BACTERIA THAT MEDIATE REFRACTORINESS TO *PLASMODIUM FALCIPARUM*

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We describe here the midgut microbial diversity to find natural candidate bacteria that could mediate refractoriness to *Plasmodium falciparum* in *Anopheles gambiae*. Bacterial communities of wild *An. gambiae* mosquitoes was recovered using a conventional culture technique, on MacConkey medium, from midguts of larval, pupal and adult stages. The MacConkey agar is a selective media that allows growth of gram negative rods from the intestine. We targeted the Enterobacteriaceae bacteria because we previously showed a correlation between the presence of this family in the mosquito midgut and *Plasmodium* infection status, as reported previously. We sequenced the 16S ribosomal RNA (rRNA) gene for the bacterial strains we isolated from wild caught mosquitoes and blasted sequences against the Silva database (release 108). The 16S rRNA sequences were aligned with reference strains and alignment used for construction of a Bionj tree to revealed the relatedness among the bacteria. Our analysis identified four families including 9 genera. *Escherichia-Shigella*, *Pseudomonas* and *Serratia* were the most frequently isolated bacteria from *An. gambiae* midguts. *Escherichia-Shigella* and *Pseudomonas* were identified in 69% and 17% of larval samples and in 58% and 11% of the pupal ones, respectively. At the adult stage, *Escherichia-Shigella* and *Serratia* had a frequency of 62% and 21% in males and a frequency of 55% and 38% in females, respectively. We report for the first time the presence of the genus *Delftia* in *Anopheles* mosquitoes, and this genus was found at low frequencies in all mosquito stages. The genus *Enterobacter* was only found in larval and adult males. Our results contrast with previous studies conducted in different malaria endemic countries, reflecting a possible effect of local factors on the adaptation of bacteria species in the mosquito midgut. However, the selective culture medium used in our study probably accounts for a part of this difference. Our data call for further investigations to verify the potential role of Enterobacteriaceae naturally occurring in the mosquito midgut for malaria control.

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COMMUNITY DIRECTED EDUCATIONAL INTERVENTION FOR MALARIA ELIMINATION IN BHUTAN: ITS EFFECT ON KNOWLEDGE, ATTITUDE AND PRACTICE

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As Bhutan moves towards elimination phase, community empowerment to take initiatives and ownership of the malaria prevention and control is very important. This would foster in maintaining malaria as priority disease within communities. Hence, this study was conducted with main objective to know the effect on knowledge, attitude and practice for malaria prevention and control as a result of community directed educational intervention by community action groups in rural malaria endemic areas of Sarpang district, Bhutan. This quazi-experimental study involved data collection from 560 households interviews (140 household per group per session), 23 indepth interviews (13 during pre and 10 during post intervention) and 21 Focus Group Discussions (12 in pre and 9 in post intervention). The study resulted in a significant improvement in knowledge ($p<0.001$), attitude ($p<0.001$) in intervention as compared to control during the post intervention survey. The practice score was significantly higher in control group ($P<0.001$), however, it should be noted that the mean score of practice in intervention group increased from 6.84+-1.26 in pre-intervention to 8.35+-1.14 in post intervention ($p<0.001$) where as it decreased from 9.19+-1.78 to 9.10+-1.98 in the control group ($p=0. 68$). When compared between pre and post, there was significant improvement in post intervention ($p<0.001$ in both attitude and knowledge score) in the intervention group. These findings were supported by the qualitative findings where most people interviewed commanded the role of community action groups and their action plans. Our study resulted in positive impact on the knowledge, attitude and practice for malaria prevention and control in malaria endemic rural areas of Sarpang district. Therefore, it is recommended for expansion of this intervention to all malaria endemic areas of Bhutan, as a sustainable means to malaria elimination in Bhutan. Further research may be conducted to see the long term effect of this intervention in malaria and other diseases of priority to the community.

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BASELINE ASSESSMENT ON THE CAPABILITY OF MICROSCOPY DIAGNOSIS TOWARDS MALARIA ELIMINATION PROGRAM IN ACEH PROVINCE, INDONESIA

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Among nearly 4.5 million of Aceh population in 2010, over three million of them lived at risk in malaria endemic areas. The Indonesian Ministry of Health plan set the objective to free Aceh from malaria in 2015. This assessment aimed to obtain baseline evidence of malaria microscopists and malaria laboratories, in order to improve malaria elimination strategies in Aceh. The baseline assessment was conducted at 23 districts in Aceh between October 2010 and July 2011. This assessment used two standardized questionaires and standardized malaria slides. These questionnaires aimed to collect personal information of microscopists and their malaria laboratories. The malaria standardized slides were used to evaluate the proficiency of all registered microscopists. The practice of malaria diagnostics and their logistic then were assessed by visiting 17 selected primary health centers. Five hundred seventy four malaria microscopists were registered at 23 districts in Aceh. These microscopists were distributed at 345 malaria laboratories, dominantly working at PHCs (69%) and hospitals (25%). Three malaria laboratories reported adequacy at 30 elements of malaria laboratories. Only six districts obtained at least 20 adequate elements, while three districts had only no more than five elements. Standardized proficiency tests revealed 413 basic/in-training, 10 advanced and 9 reference levels. No expert microscopists were found in this assessment. A standardized inventory and logistic database were not available. None of surveyed laboratories had fully operated the quality assurance program of microscopy diagnostic or rapid diagnostics. In conclusion, this publication is the first comprehensive evidence on the diagnostics capability of malaria microscopists in Aceh province, Indonesia. Their laboratories revealed as minimal infrastructure and mainly supported by basic or in-training level of malaria microscopists. Therefore, implementation of quality assurance scheme was a prerequisite to maintain high-quality microscopy in the PHCs, hospitals and field settings.

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APPRAISING LOGISTICS CHALLENGES TO BENEFICIARY ACCESS TO LONG LASTING INSECTICIDAL NETS (LLINS) IN IKORODU AND SURULERE LOCAL GOVERNMENT AREAS (LGAS) OF LAGOS STATE

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Ambitious efforts to scale-up prevention of Malaria through mass distribution of long lasting insecticidal nets is underway in Nigeria; a country that shoulders 25 percent of the Africa malaria burden. In the last two years, the National Malaria Control Program, with support from various partners, procured and distributed over 50 million LLINs in 28 states (representing over 70% of the country's total population). Despite rapidly increasing availability through mass campaigns, studies indicate that fewer than 70% of households receive the nets, while only 50.3% fulfill measures of universal coverage; the use rate is approximately 61.5%. Setbacks with other initiatives similar to these which have been linked to a number of factors, including supply chain constraints at various levels _are also evident in other malaria interventions. To understand how the beneficiaries perceive the process and to understand the logistics environment in which the activities take place, a community based coverage survey was undertaken in two LGAs (one predominantly urban and the other significantly rural). The vouchers distributed prior to the exercise do not reach many of the households; although the vouchers is the sole qualification for obtaining the LLINs, many persons obtain the LLINs without the vouchers. Again some households got more than the estimated number two LLINs) thus distorting the ability by others to obtain any. A significant number of beneficiaries are not satisfied with crowd control measures at the distribution points. Although the LLINs are issued free of cost at the point of service, there is a significant willingness to pay for the commodity amongst the populace.

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PROGRESS TOWARD MALARIA ELIMINATION IN SABANG, ACEH, INDONESIA

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Indonesia plans to eliminate malaria transmission by 2030. However, based on the present level of malaria endemicity and health infrastructure, regional datelines were differently set. Sabang municipality, historically had the highest level of malaria in Aceh aims to achieve elimination by 2013. This paper documents steps taken to re-orient Sabang's program from control to elimination, and progress towards elimination. Action toward malaria elimination was started in 2008 involving various stakeholders. We conducted vector and cases survey, developed database system, analyzed historical data temporally and spatially and carried out mass blood screening in foci area. A subset of asymptomatic cases was tested using PCR to estimate the prevalence of subpatent infections of *Plasmodium falciparum* and *P. vivax*. Despite its small size, a diverse mixture of potential malaria vectors were documented in Sabang, including *An. sundaicus*, *An. minimus, An. aconitus*, and *An. dirus.* Species were collected indoors and outdoors after 9pm. Throughout the island, 211 potential larval habits mapped. Immature stages of *Anopheles* mosquitoes were present in 29% sites. A total of 423 larvae and pupae were collected from 10 anopheles species. For baseline malaria survey, 1,446 households from six villages within two subdistricts with continuing malaria transmission were mapped using GPS and residents were interviewed. Over a two year span, the number of sub-villages with ongoing malaria transmission was reduced from 61 to 43. Coverage of malaria diagnosis and treatment, IRS, and LLINs was over 80%. Screening of 16,229 residents detected 19 positive people, for a point prevalence of 0.12%. Of the 19 positive cases, eight were detected via microscopy and 11 via PCR. All symptomatic infections were detected by microscopy. Of asymptomatic infections, five were detected with microscopy and 11 were detected with PCR. Of the 19 cases, seven were infected with *P. falciparum*, 11 were infected with *P. falciparum*, and one subject was infected with both species. The interventions documented here indicated dramatically reduction the burden of malaria in Sabang over the past seven years. High coverage of malaria diagnosis, treatment using ACT, RS and LLINs contributed to the decline of prevalence from over 4% before scale up to less than 0.2%. It is striking evidence that these interventions, proven effective elsewhere, are also effective in the context of northern Sumatra.

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ACTIVE CASE DETECTION TOWARDS MALARIA ELIMINATION: LESSONS LEARNED FROM MPUMALANGA PROVINCE, SOUTH AFRICA

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To achieve malaria elimination by 2018, South Africa's malaria control programme is reorienting towards elimination through intensified surveillance efforts with strong emphasis on Active Case Detection (ACD). Mpumalanga Province is one of three endemic provinces in South Africa

that has a functional ACD system. The National and Mpumalanga Malaria Programmes jointly evaluated the province's ACD system to identify best practices and inform ACD efforts in South Africa. The evaluation was conducted in Bushbuckridge and Nkomazi municipalities within Ehlanzeni District, the only endemic district in Mpumalanga Province, and consisted of both qualitative and quantitative data collection methods and analysis. Questionnaires were administered to all direct and peripheral ACD staff, and quantitative data was extracted from the provincial Integrated Malaria Information System (IMIS) and measured against key indicators. During ACD in Mpumalanga, case investigation teams screen contacts and neighbours of index cases using blood smears for asymptomatic individuals and rapid diagnostic tests (RDTs) for those who are symptomatic. For the period of July-December 2011, the mean number of households screened in Bushbuckridge per index case was 11 (32 blood smears and 0.2 RDTs) compared to four households screened in Nkomazi (12 blood smears and 0.6 RDTs). Although more community members were screened in Bushbuckridge per index case, only 2% of all cases (passive and active) were detected by ACD compared to 9% in Nkomazi. Blood smear positivity rate was 0.05% in Bushbuckridge and 0.63% in Nkomazi while RDT positivity rate was 11.5% and 7.2%, respectively. While there could be several interpretations of this data, it is possible that testing symptomatic community members is a more sensitive screening method as it detects considerably more cases than testing asymptomatic individuals, although further research is required to understand the most efficient and effective screening protocol in an elimination setting where vigilance is critical.

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CURRENT TREND OF MALARIA DECLINE IN ENDEMIC AREAS: SHOULD WE CELEBRATE OR WORRY?

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Malaria is undoubtfully still a major public health concern in Tanzania and worldwide in terms of morbidity, mortality and perception. For many years malaria has claimed lives of millions of people and afflicted many with body harms, psychological and financial losses. However, the current trend indicates that malaria prevalence and incidence has been declining in most parts of the world where it was usually high. In Korogwe, northeastern Tanzania malaria declined from 78.4% to 13.0% in lowlands and from 24.7% to 3.1% in highland villages between 2003 and 2008. In a neighbouring district, Muheza, prevalence declined from 85% to below 15% between 1992 and 2010. A similar trend has been observed in other parts of Tanzania and elsewhere in Africa (Rwanda, Eritrea, Ethiuopia, Kenya, Zambia, etc) although there are still pockets where malaria transmission and prevalence are still high. The exact reason for such dramatic drop has not been wholly established although most of it has been attributed to scaled-up interventions such as ITNs, IRS, ACTs, climatic changes and other unknown factors. The fact is nobody is sure why malaria is declining therefore we cannot be sure as well if it won't go up again. In areas where malaria has declined, naturally acquired immunity (NAI), which otherwise protects against malaria disease declines also. In some areas of the world, such periods of low malaria have been followed by epidemic rebounds due to diminished natural protection. In our country, malaria is declining and reasons are not clearly established. The big questions remain unanswered: are epidemic rebounds likely to occur upon malaria resurgence? And, are we ready to deal with the epidemics when (if) they occur? In this paper we explore the current situation of malaria morbidity and immunity, argue whether we should celebrate or worry about the current decline and discuss measures that should be taken to avoid or deal with worst scenarios.

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OPTIMIZING PREVENTION STRATEGIES AND PROCESSES TO REDUCE THE IMPACT OF MALARIA ON U.S. MILITARY FORCES

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Despite policies and strategies to prevent malaria, U.S. military personnel continue to contract this life-threatening disease. A series of multidisciplinary stakeholder meetings were hosted over 3 consecutive years bringing together key subject matter experts and senior leaders of the Department of Defense (DoD) to address surveillance opportunities, policies, prevention strategies and malaria diagnostics to reduce the impact of malaria on DoD service members. Initial discussions addressed challenges in malaria diagnostic testing, clinical algorithms, and medical provider training, and how these activities directly affect data quality, malaria surveillance, military readiness and patient care. Significant dialogue surrounded the dilemmas associated with chemoprophylaxis options, the lack of compliance with personal protective measures, and the need for microscopy training and diagnostic support. Further discussion focused on the lack of resource awareness and sharing across the Services and the need to improve existing education and training of deploying DoD medical personnel including guidance on diagnosis, prophylaxis, and treatment in austere field environments. As a direct result of these Malaria Stakeholder meetings, new chemoprophylaxis policies were drafted and approved; DoD research laboratories agreed to create reference microscopy slide sets as malaria diagnostic training aids, with training and education commands incorporating these training tools into their curriculum; the Armed Forces Infectious Disease Society created a malaria clinical practice guideline and diagnostic algorithm; innovative on-line educational initiatives were created to improve compliance with personal protective measures; and a research and acquisitions plan was initiated to obtain a second generation malaria rapid diagnostic test that more appropriately meets the DoD's field requirements. Stakeholder meetings with breakout sessions and subsequent committees are able to provide tremendous progress and address multiple programmatic gaps to provide better strategies, resources, and care to service members in the DoD.

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MALARIA FOCAL SCREEN AND TREAT IN LUSAKA DISTRICT, ZAMBIA: A WAY FORWARD FOR SURVEILLANCE TO ACHIEVE THE GOAL OF MALARIA ELIMINATION

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The Zambia Ministry of Health (MoH) has a goal of malaria elimination in five geographic areas by 2015. New surveillance and intervention

strategies are being rolled out to support this goal. In Lusaka, Zambia's capital city, indoor residual spraying, improvements in malaria surveillance and case management training have contributed to a marked reduction in malaria cases and antimalarial consumption. Further, the low malaria prevalence has shown the area to be a candidate for malaria elimination. In March 2011, the National Malaria Control Center and Lusaka District Health Office began a focal screen and treat (FST) activity where laboratory-confirmed malaria cases are followed-up in the community through testing and treating of residents of the index household and surrounding households. The FST activity is occurring in ten catchment areas in Lusaka district. MoH personnel including an environmental health technician, a nurse and community health workers respond to eligible cases. During the focalized response, residents of the index house and nine neighboring households are screened and treated for malaria, bednets are provided, and malaria information is shared. From March 2011 through March 2012, 104 index case patients (reporting no recent travel or malaria) were identified by the ten clinics. Response teams screened 3,419 individuals for malaria using rapid diagnostic tests. Of these, 58 (1.7%) individuals were found to be positive with 28 reporting no recent travel or malaria indicating probable local transmission. All other RDT-positive individuals reported previous malaria within one month (potential false positive by RDT) or recent travel outside of Lusaka. This system is the first malaria surveillance and response system to be embedded within the MoH and has been shown to be sustainable and cost-effective with the MoH assuming oversight and expense. Analysis of its capability to provide sensitive and specific information on progress to malaria elimination is timely, especially as the system is being expanded to other areas in Zambia.

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RAPID MAPPING OF SEASONAL MALARIA TRANSMISSION RISK FOR STRATEGIC ELIMINATION PLANNING IN SWAZILAND

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As successful malaria control programs move towards elimination, they must identify residual transmission foci, focus on both asymptomatic and symptomatic infections, and manage importation risk. High spatial and temporal resolution maps of malaria risk can support all of these activities, but new approaches are required to provide accurate case-based risk maps for very low prevalence countries like Swaziland, where fewer than 500 cases were reported in 2011. Household locations and travel histories of confirmed malaria patients were recorded through routine surveillance by the Swaziland National Malaria Control Programme in 2011. Household locations with locally-acquired infections were compared against a random set of background points with respect to variables related to environment, population density, vector control, and distance to the households of imported cases. The regression tree classification approach Random Forest was used to generate maps predicting the probability of a locally-acquired case at 100 m resolution across Swaziland during the high and low transmission seasons. Results indicated that case households during the high transmission season tended to be located at lower elevations, closer to stream channels, in more sparsely populated areas, with higher rainfall and lower temperature than random background points (all $p<0.01$). Similar differences were evident during the low transmission season, but environmental variables like distance to stream channels and water bodies were no longer significantly different, while low season case households were located significantly nearer to those of imported cases($p=0.02$). Maps from the fit models suggested better predictive ability during the high season. The rapid, high-resolution mapping approaches described here appear useful for helping elimination programs understand the epidemiology of a disappearing disease, direct interventions in response to evidence-based measures of risk, and ensure that the impact of limited resources is maximized to achieve and maintain malaria elimination.

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MECHANISMS OF A MOSQUITO-BASED MALARIA TRANSMISSION-BLOCKING VACCINE

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Malaria continues to be a tremendous global health burden, yet no vaccine is currently available. Transmission-blocking vaccines (TBVs) that prevent sporogonic development of parasites within *Anopheles* mosquitoes, and the subsequent cascade of human infections, are a potentially effective approach. The highly conserved *Anopheles gambiae* alanyl aminopeptidase (AnAPN1) was recently identified as a putative but critical mosquito midgut ligand for *Plasmodium* ookinetes. Antibodies against an N-terminal fragment (NT135APN1) in rabbits demonstrated cross-species inhibition, preventing development of *P. falciparum* in *An. gambiae* and *P. berghei* in *An. stephensi* in laboratory models. The ability of α-AnAPN1 antibodies to recognize orthologous mosquito midgut aminopeptidase antigens and block multiple *Plasmodium* species implies significant utility as a pan-malaria TBV candidate. We report here on AnAPN1 immunogenicity, efficacy, and mechanisms of inhibition of anti-AnAPN1 antibodies in multiple animal models. Immunization of inbred and outbred mice and non-human primates with NT135APN1 elicited potent transmission-blocking antibody titers against *P. falciparum* (NF54) in standard membrane feeding assays, while rabbit α-AnAPN1 antibodies inhibited field isolates of *P. falciparum* and *P. vivax* in *An. gambiae* and *An. dirus*, respectively. Synthetic peptide-based ELISAs and comparative immunoblotting suggest that transmission-blocking activity of α-AnAPN1 antibodies against *P. falciparum* in each of these animal models is conferred by binding a single conserved predicted linear B cell epitope. Antibodies from mice immunized with peptide corresponding to this epitope exhibited cross-reactive recognition of recombinant NT135APN1 and native AnAPN1 and inhibited *P. falciparum* oocyst development in *An. gambiae*. Finally, α-AnAPN1 antibodies appear to inhibit *Plasmodium* transmission by binding ookinetes, either directly or indirectly, and not by inhibiting enzymatic activity of AnAPN1. These data provide initial proof-of-principle for the plausibility of a mosquito-based pan-malaria TBV.

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DIFFERENTIAL ACTIVATION OF DENDRITIC CELLS BY NANOPARTICLE-COATED *PY*MSP-1 DNA VACCINE USING DIFFERENT ROUTE OF DELIVERY

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In malaria DNA vaccine development, there exists a critical need for additional delivery vehicles which may facilitate targeting and/or controlled release of antigen to antigen presenting cells such as dendritic cells. We have previously shown the immuno-stimulatory and protective effect of nanoparticle (NP)-coated *Plasmodium yoelii* merozoite surface protein 1 (PyMSP-1) plasmid with high level of IL-12 production. It has also being reported that γ-PGA NPs were preferentially internalized by DCs and induced the production of IL-12. Here we attempted to investigate the *in vivo* stimulatory effect of NP-coated plasmid on dendritic cells by analyzing the expression of antigen presentating molecule MHC class II, co-stimulatory molecules and cytokines production in group of mice immunized with NP-coated and naked MSP-1 plasmids. Groups of six week old female C57BL/6 mice were immunized either intraperitoneally (i.p.) or subcutaneously (s.c.), with 100 µg/mouse of either NP-coated-plasmid DNA (pVR1020-MSP-1/PEI/γ-PGA) or naked plasmid DNA (pVR1020-MSP-1). Mice were prime-immunized at day 0 and two subsequent boosters at three weeks intervals. Two weeks after the last boost, IgG and its subtype antibody responses were assessed by ELISA from the individual sera. The mice were then sacrificed, and freshly isolated lymph node and splenic cells were stained to analyze the proportion of T cells and various activated DC markers by flow cytometry. Cytokine (IL-12 and IFN-γ) levels were measured in the supernatants of antigen stimulated lymph node and spleen cells and sera from immunized mice. We observed an increased proportion of activated DCs and expression of CD40 in the group of mice immunized with NP-coated MSP-1 as compared to naked plasmid. CD80 and CD86 co-stimulatory molecules were increased in the coated group immunized by s.c. and i.p., respectively. Higher levels of IL-12 and INF-γ production were induced in splenocyte and lymph node cells cultured supernatants from NP-coated MSP-1 vaccinated mice across the two routes of administration. It is apparent here that DC activation, CD40 expression and IL-12 production following rMSP-1 stimulation, were significantly induced in NP-coated group across the two route of delivery. These data, indicated that nanoparticle-coated PyMSP-1 DNA vaccine induced activated DCs either with CD80 or CD86 and those activated DCs produced IL-12 when stimulated by rMSP-1.

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PROTEIN GLYCOSYLATION AND IMMUNOGENICITY OF DNA VACCINES

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Plasmodium falciparum 25kDa protein (Pfs25), a post-fertilization zygote-ookinete surface protein is a leading transmission blocking vaccine candidate against malaria. In the past our lab has shown functional immunogenicity as well as transmission blocking potential of DNA vaccines encoding Pfs25 in mice and nonhuman primates. Pfs25 contains 3 putative N-linked glycosylation sites, although malarial proteins are usually not extensively glycosylated. Using DNA vaccine platform and *in vivo* electroporation (EP), we are investigating the effect of codon optimization and N-glycosylation site mutations on vaccine immunogenicity parameters in mice with the aim of optimizing the immunogenicity. We compared three DNA plasmid constructs- wild type (WT), codon optimized (SYN) and codon optimized with N-glycosylation site mutations (MUT). By mammalian HEK293T cell transfection and western blotting we identified differences in patterns of protein production between the groups. SYN DNA produced a stronger signal than WT. MUT DNA produced a sharper, smaller size band than WT and SYN, resulting from lack of glycosylation of the expressed protein. We confirmed that the difference was in fact due to glycosylation by treatment with tunicamycin (TN), an N-glycosylation inhibitor. Immunization studies in mice (N=5 per group) were done using three concentrations of each DNA plasmid (25mg, 2.5mg and 0.25mg per dose) with EP and a 25mg dose of each without EP. Our studies suggested improved immunogenicity with EP in contrast to intra-muscular injections alone and showed higher immunogenicity of codon optimized SYN groups when compared to WT DNA. Interestingly, immunization with 25ug MUT DNA with EP produced antibody titers that were twice as high (1:512,000) compared with SYN (1:256,000) and four fold higher compared with WT (1:128,000). Even at 0.25mg concentration, MUT DNA produced titers at 1:128,000 while the titers were 1:16,000 with SYN and with WT DNA ELISA titers were not significant. Our data show that mutating putative glycosylation sites affects the final antigenic product and suggests that the presence of carbohydrate side chains adversely affects the immunogenicity of Pfs25. Further studies using membrane-feeding assays are pending to detect whether these mutations improve the functional immunogenicity of the vaccine.

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VAR2CSA DUFFY BINDING LIKE (DBL) DOMAINS AND NON-SPECIFIC IGM BINDING IN THE ACQUISITION OF NATURAL IMMUNITY TO PREGNANCY-ASSOCIATED *PLASMODIUM FALCIPARUM* MALARIA

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Plasmodium falciparum malaria throughout history has proved to be a significant menace to human health and pregnancy malaria has been linked to severe consequences such as increased maternal anaemia, low birth weight and infant mortality. *P. falciparum* parasites express members of the as reported previously Erythrocyte Membrane Protein-1 (PfEMP1); protein family on the surface of infected erythrocytes (IEs), as reported previously. These PfEMP1 proteins act as ligands binding to a number of different human vascular host receptors, which allow IEs to sequester in various tissues and escape destruction in the spleen, as reported previously. PfEMP1 proteins are therefore important targets of acquired protective immunity, which is thought to be mediated mainly by specific IgG antibodies, as reported previously. In addition to being targets of specific IgG, several PfEMP1 variants can bind natural IgM, as reported previously. However, the biological significance of this non-specific binding is unclear, as reported previously. Whether PfEMP1 is also significantly targeted by non-specific IgM is relatively unknown and hence the need for this research. The aim of this study is to identify the role of VAR2CSA-nonspecific IgM in acquired immunity to *P. falciparum* pregnancy associated malaria. Plasma samples from 100 pregnant Ghanaian women of varying age, gestational age and parity have been purified by the Dynabeads immunoprecipitation method for IgG and IgM on Mannan Binding and grouped into three (unpurified plasma containing IgG and IgM, purified IgG from plasma and purified IgM from same plasma). The purified and unpurified immunoglobulin (IgG and IgM) levels to VAR2CSA Duffy Binding Like (DBL) domains from the pregnant women are been

quantified using commercially available ELISA kits. This study will provide data on the role of PfEMP1-nonspecific IgM in acquired immunity to *Plasmodium falciparum* malaria in pregnancy which will be useful in the development of an effective vaccine against pregnancy associated malaria.

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MECHANISTIC BASIS OF *PLASMODIUM FALCIPARUM* NEUTRALIZATION BY ANTI-RH5 ANTIBODIES

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Vaccines against asexual blood-stage of *Plasmodium falciparum* have not achieved clear efficacy in clinical trials. Challenges include antigenic polymorphism, recombinant antigen production, and achievement of high antibody titres without excessive reactogenicity. We have previously shown that vaccines based upon the full-length reticulocyte-binding protein homologue 5 (RH5) induce antibodies which neutralise all tested laboratory-adapted parasite strains. More recently, we have found that neutralisation of recently-isolated parasites by anti-RH5 is more potent than with anti-AMA1 antibodies, and have identified synergistic effects of mixtures of anti-RH5 IgG with other polyclonal antisera. We hypothesised that blockade of the interaction of RH5 with its receptor basigin was likely to be a mechanism of action of anti-RH5 antibodies. We have found that vaccine-induced polyclonal anti-RH5 serum is capable of blocking this interaction, as well as merozoite attachment to erythrocytes. We have also raised a panel of RH5-specific monoclonal antibodies: those which block the RH5-receptor interaction are capable of neutralising parasites. Minimal linear epitopes recognised by these antibodies were mapped, and are likely to be within or close to RH5's receptor binding site. Further data relating to the mechanism of anti-RH5 antibody neutralisation of parasites will be presented.

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GIA, ELISA AND PROTECTION IN A *PLASMODIUM KNOWLESI* MODEL

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Plasmodium falciparum apical membrane antigen 1 (PfAMA1) is a leading blood stage vaccine candidate currently undergoing phase II clinical studies. Here, we used *P. knowlesi* and rhesus macaque as a model (1) to test efficacy of AMA1 and (2) to identify correlates of protection. PkAMA1 was produced and purified using two chromatographic steps simlar to methodologies used for clinical grade PfAMA1. T two wo groups of six rhesus macaques were immusied on day 0,28, 56 with 50 µg Pk or Pf AMA1 in CoVaccine HT adjuvant. Monkeys were challenged on day 70 with P. knowlesi H strain iRBC i.v re-boosted (day 202). Rhesus were re-challenged on day 217 and later on day 450. Parasitaemia were monitered daily after each challenge. ELISA and GIA were performed using standard protocols.Expression of PkAMA1 yielded a highly pure conformational intact protein. One of six rhesus monkeys was able to control parasitaemia, upon blood stage challenge with Pk H-strain. Four out of the remaining 5 showed a delay in parasite onset that correlated with ELISA and GIA titres. Following the second challenge, four of the six monkeys were able to control parasitaemia, one had a delayed onset of parasitaemia, while all control animals became parasitaemic. Upon the third challenge 5 out of 6 PkAMA1 vaccinated animals were able to completely control parasitaemia. High GIA levels correlate with protection (Spearman's Rho = -0.93, p =0.008. This study shows that: i) Heterologously expressed PkAMA1 can protect against blood stage challenge ii) Protection improves after challenge-boost and iii) Functional antibodies levels correlated inversely with the day of onset.

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IMMUNOGENICITY AND PROTECTIVE EFFICACY OF *PLASMODIUM FALCIPARUM* MSP1-42 VACCINE FORMULATIONS AGAINST *PB-PF*M19 TRANSGENIC PARASITE INFECTIONS IN MICE

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Merozoite surface protein (MSP) 1 is essential to the *Plasmodium falciparum* parasite life cycle. Many preclinical and seroepidemiological studies have demonstrated that antibodies to MSP1 can either prevent or control blood stage infections making the antigen a relevant target for a malaria vaccine. MSP1 vaccine formulations evaluated in human subjects, however, have not demonstrated broad efficacy; thus, evaluation of alternate vaccination platforms is necessary. Currently, there is no preclinical correlate of immunity for blood stage vaccines. Recent findings have demonstrated that *in vitro* growth inhibition assays (GIA) do not fully capture the biological function of MSP1-specific antibodies. For this study, we have employed a PfMSP1-19 transgenic *P. berghei* parasite model to measure the anti-parasite activity induced by immunization with either recombinant PfMSP1-42 protein adjuvanted with Montanide ISA720 or PLGA-beads of different sizes coated with PfMSP1-42 in the presence or absence of Monophosphoryl lipid A. Mice challenged with transgenic PfMSP1-19 *P. berghei* were screened daily for the presence of parasitemia by *qRT-PCR* (Days 1-5, and 15) and flow cytometry (Days 5-15). Humoral immune responses were characterized by measuring MSP1-specific antibody concentrations, isotype, avidity and evaluating the functional activity of these antibodies in GIA assays. Surprisingly, no correlation between GIA activity and protective efficacy was observed in the challenge model for these formulations, with formulations with the highest GIA activity not necessarily conferring protection. Both cellular and humoral immune responses differed depending on the size of beads used. However, PLGA-coated antigen-uptake on mouse dendritic cells did not predict formulations with the highest efficacy. The study demonstrates the advantages of the transgenic PfMSP1-19 *P. berghei* challenge model for evaluating MSP1-based vaccines and underscores the importance for evaluating the activity of antibodies *in vivo*.

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PROTECTION AGAINST A *PLASMODIUM BERGHEI* SPOROZOITE CHALLENGE INFECTION WITH A *P. FALCIPARUM* CELTOS VACCINE ADJUVANTED WITH GLA-SE

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Second generation malaria vaccines are currently being identified with the help of reverse vaccinology, which takes advantage of genome- and proteome-based antigen discovery. For pre-erythrocytic malaria vaccines, targeting immune responses to antigens expressed on sporozoites can impact the ability of parasites to migrate to the liver and/or infect hepatocytes. The *Plasmodium* Cell-traversal protein for ookinetes and sporozoites (CelTOS) plays an essential role in parasite movement in both mosquitoes and vertebrates and is required for successful infection. We previously demonstrated that an *E. coli*-expressed CelTOS protein from *P. falciparum* adjuvanted with the water-in-oil adjuvant Montanide ISA

720 is protective in Balb/c mice challenged with heterologous *P. berghei* sporozoites. Both cellular and humoral immune responses contributed to protection mediated by this CelTOS vaccine. In an effort to adapt the vaccine to the clinic, we tested in this mouse model an adjuvant that has previously been used in humans and that lacks the toxicity seen with Montanide ISA-720. The current study compares PfCelTOS adjuvanted in different amounts of the synthetic TLR-4 agonist Glucopyranosyl Lipid A (GLA) mixed in a stable emulsion (SE) and tests their potency to induce protective humoral and cellular responses in Balb/c mice against a *P. berghei* sporozoite challenge. These vaccines enhanced both humoral and cellular immune responses, which were characterized by preferential elevation of the IgG2a antibody isotype, functional antibody activity against sporozoites, and the induction of strong Th1-type immune responses. These findings provide the pre-clinical support for further evaluation of this vaccine formulation in a Phase 1 clinical study assessing safety, immunogenicity and efficacy in U.S. naïve subjects.

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PLASMODIUM YOELLI PARASITES AND BACILLUS CALMETTE GUERIN (BCG) VACCINE: FRIENDS OR FOES

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Although BCG is used worldwide as a vaccine against TB, its effectiveness in preventing tuberculous disease remains controversial. However, epidemiologic studies have indicated that BCG provides other general health benefits to vaccinees including the reducing the impact of asthma, leprosy, and possibly malaria. To evaluate whether BCG immunization protects against malarial parasitemia, mice were vaccinated with BCG and then challenged 2 months later with *Plasmodium yoelii* parasites. Significant decreases in parasitemia were seen in BCG vaccinated mice relative to naïve controls. To identify immune molecules that may be associated with the BCG-induced protection, gene expression was evaluated by RT-PCR in BCG-vaccinated mice at day 0, 1, 5, 9 and 90 after the *P. yoelii* infection. The expression results showed that i.) BCG immunization induces the expression of at least 15 genes including the anti-microbial peptides CAMP, lactoferrin, eosinophil peroxidase, and eosinophil major basic protein; ii) an active *P. yoelii* infection suppresses the expression of important immune response molecules such as iNOS and IFN-γ; and iii) the *P. yoelii*-induced suppression of specific genes (ie., iNOS) is greatly reduced in BCG-vaccinated mice. To validate the gene expression data, we demonstrated that lactoferrin treatment decreases the level of *P. yoelii* infection in mice and BCG vaccination does not impact the course of malaria infection in iNOS knockout mice. Overall, our study suggests that BCG vaccination induces the expression of the non-specific immune molecules including antimicrobial peptides which may provide overall health benefits by limiting infections of unrelated pathogens such as *Plasmodium* parasites.

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SAFETY OF AN INVESTIGATIONAL MALARIA VACCINE BASED ON THE $MSP1_{42}$ FVO ALLELE ADJUVANTED WITH GLAXOSMITHKLINE'S AS01B ADJUVANT SYSTEM IN WESTERN KENYA

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The Merozoite Surface Protein-1 (MSP-1) is a promising malaria vaccine candidate antigen, the 3D7 allele of which has undergone evaluation in multiple clinical trials at WRAIR and KEMRI/ Walter Reed Project in combination with a GlaxoSmithKline Biologicals proprietary adjuvant system. A first-in-human Phase 1a dose escalation trial of the $MSP1_{42}$ FVO allele was conducted in malaria-naïve adults at WRAIR and was found to have a good tolerability profile. A Phase 1b trial using a 50 µg dose of the antigen with adjuvant system AS01B was conducted in Western Kenya. This was a randomized, controlled and double-blind with Rabipur® (Norvatis) rabies vaccine as the comparator. It was designed to enroll 30 adults: 20 to the $MSP1_{42}$/AS01B arm and 10 to the rabies (Rabipur®) arm. The vaccinations were given a month apart, into the deltoid muscle of the non-dominant arm. Follow up was conducted for safety, determination of antibody fine specificity, and functional immunogenicity; the latter being evaluated via pLDH Growth Inhibition Assay (GIA). The vaccine was found to be safe with only one subject experiencing a grade 3 adverse event and no SAEs reported. Vaccination produced high levels of anti-$MSP1_{42}$ antibodies that were long lived. Here we report, in detail, the safety and immunogenicity results from this study.

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MATHEMATICAL MODELLING OF THE RELATIONSHIP BETWEEN RTS,S VACCINE-INDUCED ANTIBODY LEVELS, T CELL MEDIATED RESPONSES AND PROTECTION AGAINST *PLASMODIUM FALCIPARUM* INFECTION IN MALARIA-NAÏVE ADULTS

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The RTS,S candidate pre-erythrocytic malaria vaccine has demonstrated immunogenicity and efficacy against *Plasmodium falciparum* infection and clinical disease in phase 2 human challenge trials, and has been demonstrated to induce high levels of antibodies and robust CD4+ T cell responses targeting the circumsporozoite protein (CSP). Using data from 138 malaria naïve adults, we sought to characterise the relationship between antibody levels, T cell responses and protection from *P. falciparum* infection using a biologically-motivated mathematical model of infection relating the sporozoite load in an infectious bite to the probability of infection and the time of onset of blood-stage parasitemia. Dose-response curves were used to estimate the relationship between anti-CSP antibody titres and the number of CSP-specific T cells and the efficacy of the vaccine in blocking sporozoite infection. Both anti-CSP antibody titres and CSP-specific T cells were identified as immunological

correlates of protection, with 50% protection from infection being conferred either by a vaccine-induced anti-CSP antibody titre ≥ 253 (95% CI, 154 - 482) µg/mL, or by CSP-specific T cells at a frequency ≥ 3235 per million (95% CI, 1696 - 18465). Adjuvant formulation did not have a direct effect on vaccine efficacy, but contributed to protection only by increasing the magnitude of induced immune responses. Based on the delay in time to onset of parasitemia in vaccinated volunteers, RTS,S is estimated to cause a 98.3% (95% CI, 97.3% - 99.1%) reduction in the number of merozoites emerging from the liver to initiate blood-stage infection. In vaccinees who developed a *P. falciparum* infection, a small number of parasites (often a single sporozoite) are responsible for breakthrough infections.

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PHASE 1 TRIAL WITH CHALLENGE TO ASSESS THE SAFETY, IMMUNOGENICITY, EFFICACY AND BIOMARKERS OF PROTECTION IN MALARIA-NAÏVE ADULTS OF IMMUNIZATION VIA MOSQUITO BITE WITH RADIATION-ATTENUATED *PLASMODIUM FALCIPARUM* SPOROZOITES (IMRAS)

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In the early 1970s, it was shown that radiation-attenuated *Plasmodium falciparum* sporozoites (*Pf*RAS) delivered via mosquito bite to malaria-naïve subjects conferred sterile protection by inducing an immune response targeting the pre-erythrocytic stages of the parasite. Despite years of effort, the immunological mechanisms and antigens targeted by the protective immunity have not been fully delineated, although CD8+ T cells recognizing infected hepatocytes likely play a key role. With the recent demonstration that non-attenuated *Pf* sporozoites, administered via mosquito bite with chloroquine coverage to prevent blood stage infection, can likewise confer potent sterile immunity in humans, as reported previously, the search for protective immune responses and target antigens has intensified. The IMRAS trial will apply systems biology to identify processes underlying *Pf*RAS immunization, focusing on both innate and acquired immunity. 24 malaria-naïve subjects will be immunized with *Pf*RAS via mosquito bite and challenged with wild-type sporozoites to ascertain protection. Samples (PBMCs, RNA, plasma) will be collected before, during and after immunization and challenge. Transcriptional profiles, plasma chemokine and cytokines, immune cell phenotypes and functions, humoral inhibition of sporozoite invasion and liver stage development will be determined. These data will be integrated using systems approaches to identify correlates of protection. Through this study, the nature of pre-erythrocytic stage protective immunity as well as the targeted antigens will be characterized, accelerating vaccine development.

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EFFICACY OF RTS,S MALARIA VACCINES AND CAUSES OF HETEROGENEITY: POOLED ANALYSIS OF INDIVIDUAL PARTICIPANT DATA FROM PHASE 2 TRIALS

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The efficacy of RTS,S/AS01 as a vaccine for malaria is being tested in a phase III clinical trial. Early results demonstrate significant, but partial, protection against clinical malaria and severe malaria. In order to predict the efficacy of vaccination in diverse settings, we need to understand the impact of covariates such as transmission intensity, age at vaccination, and bednet use on vaccine efficacy. Furthermore, there have been no definitive comparisons of the impact of adjuvant choice on efficacy (i.e. AS01 vs AS02). We conducted an individual participant pooled analysis of the Phase II clinical trials with data on efficacy. Data were analysed from 11 different sites in Africa, including 4,453 participants. We examined heterogeneity in vaccine efficacy by estimating the interactions between covariates and vaccination in pooled multivariable Cox regression and Poisson regression analyses. Vaccine efficacy against multiple episodes of clinical malaria was lower at increasing transmission intensity (Incidence Rate Ratio (IRR) =2.47, 95% Confidence Interval (CI) 1.45 to 4.21, p=0.001 for children at 50% parasite prevalence compared with 10%), for RTS,S/AS02 compared with RTS,S/AS01 (IRR=2.30, 95%CI 1.54 to 3.44, p<0.0005). Vaccine efficacy was higher for 3 year old children compared with 5mth old children (IRR=0.92, 95%CI 0.85 to 0.99, p=0.038). Estimated vaccine efficacy declined significantly over time, with estimated efficacy against clinical malaria approaching 0% by 3 years. There was no significant variation in efficacy against clinical malaria by bednet use, or by gender. We conclude that the outcomes following vaccination will not be predicted accurately based on a pooled efficacy figure alone. The local transmission setting, age at vaccination, and the duration of risk must also be considered.

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IMMUNIZATION WITH *PLASMODIUM FALCIPARUM* SPOROZOITES UNDER CHLOROQUINE PROPHYLAXIS

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Previously we demonstrated that immunization of healthy volunteers under chloroquine prophylaxis with *Plasmodium falciparum*-infected mosquito bites (ChemoProphylaxis and Sporozoites, CPS immunization) induces complete protection against a homologous malaria challenge infection. The induction of parasite-specific pluripotent effector memory T cells producing interferon-γ and interleukin-2 associated with protection. In a series of three consecutive clinical trials, we investigated in more detail the nature of the protection induced by CPS immunization. We first performed a dose titration of *P. falciparum*-infected mosquitoes to determine the minimum protective dose. In a group of 24 immunized volunteers, 17 were protected and 7 unprotected against a challenge. This

gives us the opportunity to now more closely investigate the association between cellular immune responses (measured by *in vitro* re-stimulation of peripheral blood mononuclear cells) and protection. Next, we investigated in a second trial whether CPS immunization protects against erythrocytic stages or primarily against pre-erythrocytic stages of the parasite. When CPS-immunized volunteers were challenged either with infected mosquito bites or by intravenous administration of *P. falciparum* infected erythrocytes, we found no protection against a pure blood stage challenge. This clearly shows that protection by CPS immunization is mediated be pre-erythrocytic immunity. Finally, we asked whether CPS immunization also protects against mosquito challenge with a heterologous strain. This is important given the large variation of different strains present in malaria-endemic areas. Therefore, we are currently re-challenging CPS immunized volunteers from the first trial and five naïve control volunteers with a genetically distinct *P. falciparum* strain. We will discuss the results of these studies and their important implications for understanding protective immunity against *P. falciparum.*

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PRODUCTION OF A MALARIA TRANSMISSION BLOCKING VACCINE CANDIDATE PFS25 IN *PICHIA PASTORIS* WITHOUT AN AFFINITY TAG FOR HUMAN CLINICAL STUDIES

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Approximately one-half of the world's population lives in areas exposed to the malaria parasite *Plasmodium falciparum* resulting in an estimated million deaths annually, 85% of which occur in children under 5 in sub-Saharan Africa. The development of a transmission blocking *P. falciparum* malaria vaccine is considered critical for future control measures of elimination and eradication. To this end, a malaria transmission blocking vaccine against an ookinete protein, identified as Pfs25 is being pursued which targets the malaria parasite as it reproduces in the mosquito's gut. Pfs25 contains 4 epidermal growth factor-like domains comprised of a total of 11 disulfide bonds. Human antibodies generated against an experimental *Pichia pastoris* (Pp) produced Pfs25(H) vaccine when taken up by the mosquito in a standard membrane feeding assay inhibit parasite development and subsequently block mosquito infectivity. To facilitate development beyond initial phase I and II testing, a second generation production clone has been produced in order to remove 14 heterologous amino acids including a HIS_6 affinity tag. The second generation PpPfs25(M) production clone was generated by transforming *Pichia* GS115 with a *P. pastoris* protein disulfide isomerase co-expression vector into which the synthetic Pfs25(M) gene was cloned such that the secreted PpPfs25(M) protein contained no heterogeneous amino-acids. Fermentation development evaluating induction temperature, pH and methanol feed rates in defined media using a Box-Benkhen surface response design of experiment model (total of approximately 35 fermentation runs) in 5 liter fermenters yielded product at over 1 gram/liter supernatant of PpPfs25(M) material assessed by an analytical ion-exchange-HPLC method. A robust fermentation process was established and performed at pilot-scale in a 60L working volume fermentation and yields were greater than 1 gram/liter supernatant. PpPfs25(M) has been purified using standard scalable chromatographic methods, characterized biochemically and biophysically, and shown to be functionally similar to Pfs25(H) using the standard membrane feeding assay. Production of bulk material following cGMP is ongoing for future clinical studies.

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BLOOD STAGE MALARIA VACCINES: ASSESSMENT OF CLINICAL ENDPOINTS FOR VACCINE TRIALS

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Vaccines directed at the blood-stage or asexual cycle of malaria have the potential to reduce the morbidity and economic burden of malaria disease encountered worldwide each year. Mathematical models of human malaria enable the impact of such vaccines to be quantified and assessed. Although some models have examined the effect of vaccines in preventing disease at the population level, mechanistic within-host models of parasitaemia are needed to understand how interactions between vaccines and immune responses modify infection dynamics and consequently disease burden, incidence and transmission. We proposed a new mechanistic model of *Plasmodium falciparum* and the associated immune responses within a human host to examine the potential effect of blood stage vaccines on infection dynamics and parasite densities with several putative mechanisms of action. Via these results and similar investigations with other published models of *Plasmodium falciparum* dynamics we found that the effect of these vaccines could be surprisingly small, depending on how blood-stage vaccines affect antibody production. Our results also suggest that efficacy criteria and endpoints for clinical trials of malaria vaccines be carefully re-examined, as the some endpoints may incorrectly reject effective vaccines. This work emphasises the importance of using mathematical models to assess the design of clinical trials and their endpoints.

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QUALIFICATION OF STANDARD MEMBRANE-FEEDING ASSAY WITH *PLASMODIUM FALCIPARUM* MALARIA FOR DEVELOPMENT OF TRANSMISSION-BLOCKING VACCINES

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Transmission-blocking vaccines (TBV) are of increasing interest and a strong assay will support TBV development. To address this, we have attempted to qualify the standard membrane-feeding assay (SMFA) in which the transmission-blocking (TB) activity of test antibodies is evaluated using cultured *Plasmodium falciparum* gametocytes and *Anopheles* mosquitoes. According to the ICH Harmonised Tripartite Guideline Q2(R1), up to seven characteristics need to be considered for assay validation depending on the type of assay. Of these seven, we decided to qualify the SMFA in terms of: Precision (specifically, Repeatability and Intermediate Precision), Linearity, Specificity and Range. We generated a quantity of 4B7 monoclonal antibody (mAb), which has TB activity. The 4B7 mAb was tested over multiple runs at several concentrations to determine the range to use for qualifying the assay. In the qualification test, four concentrations of 4B7 mAb were tested in triplicate in three different experiments to evaluate the Precision, Linearity and Range. For the test of Specificity, IgG from normal mouse sera was prepared and tested by SMFA with and without addition of 4B7 mAb. We found; 1) Intra- and Inter-assay variability of % inhibition in oocyst density were relatively comparable and clearly depended on the concentration of 4B7 mAb (lower concentration showed significantly higher variability), 2) 0.75 mg/ml of normal mouse IgG did not significantly change the % inhibition of 4B7 mAb. In addition, we generated a model to improve the assay and analytical methods for future studies. The model predicts; 1) if the same total number of mosquitoes are dissected, it is better to dissect smaller numbers of

mosquitoes from multiple groups rather than larger numbers from one group in terms of the variability, 2) % inhibition calculated using mean has less variance than that using median, 3) using log-transformed ratio of control and test may lessen the effect of concentration on variance in % inhibition. The qualification and improvement of SMFA should accelerate future TBV development.

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EVALUATION OF FIELD FEEDING ASSAYS AND TRANSMISSION RESERVOIR IN PREPARATION FOR TRANSMISSION BLOCKING VACCINE FIELD TRIALS

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Transmission blocking vaccine (TBV) is an integral part of malaria control and eradication. In preparation for a Phase 1b trial testing a Pfs25-based TBV, our current study in Mali aims to survey gametocyte carriage rates at a trial site, and to develop and standardize assay methods for evaluation of TBV efficacy. A total of 250 volunteers, from 3 months to 50 years of age, were recruited for monthly surveys of asexual and sexual parasite carriage. Participants in older age groups participated in feeding assay studies using lab-reared mosquitoes free of known transmissible viruses. Direct Skin Feeds (DSF), where mosquitoes were allowed to feed directly on volunteer's legs, and Direct Membrane Feeds (DMF), where mosquitoes feed on volunteer's blood through a membrane feeder, were conducted to establish baseline infectivity and to standardize assay methods. Volunteers participating in DSF were followed closely, and there were no feed-related adverse events. In DMF, multiple samples were tested to compare mosquito infectivity using whole blood, washed blood reconstituted with autologous plasma, and washed blood reconstituted with a naïve serum pool from U.S. volunteers. The mosquito feeding rate and the baseline infection rate were higher with DSF compared to DMF methods, and critical parameters affecting mosquito feeding rate and the baseline infection rate with DMF were identified, optimized, and standardized. The presence of natural transmission blocking activity in volunteers' plasma was demonstrated by partial restoration of mosquito infectivity in DMF after replacing autologous plasma with naïve sera, and by Standard Membrane Feeding Assays conducted in the US. In conclusion, DSF is a safe and suitable *ex vivo* assay closest to natural setting for evaluation of TBV efficacy. DMF is also a valuable assay for evaluation of antibody-specific effect, and may replace DSF once its baseline mosquito infectivity is improved through assay standardization.

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OVERCOMING ALLELIC-SPECIFICITY BY IMMUNIZATION WITH 5-ALLELIC FORMS OF *PLASMODIUM FALCIPARUM* APICAL MEMBRANE ANTIGEN 1

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Apical Membrane Antigen 1 (AMA1) is one of the leading vaccine candidates, but the allelic polymorphism is a stumbling block for vaccine development. Previously we have shown global set of AMA1 haplotypes could be grouped into 6 genetic populations. Using this information, six recombinant AMA1 proteins representing each population were produced and characterized. Rabbits were immunized with either single or mixture of them (4, 5 or 6 mixtures). Antibody levels were measured by ELISA, and purified IgG from each rabbit was used for Growth Inhibition Assay (GIA) with 12 different clones of parasites (total of 108 immunogen-parasite combinations). Levels of antibodies to all 6 AMA1 proteins were similar when they were tested against homologous antigens (e.g., anti-AMA1-3D7 antibody was tested with AMA1-3D7 ELISA antigen). When % inhibitions in GIA were plotted against ELISA units measured with homologous AMA1 (e.g., antibody levels of all samples were measured using AMA1-3D7-coated ELISA plates, and tested by GIA with 3D7 parasites), all data points followed a sigmoid curve regardless of immunogen. Homologous combinations showed higher antibody titers than heterologous combinations in ELISA, and higher % inhibition in GIA. In homologous combinations, there were no differences in % inhibition between single and mixture (i.e., one of the mixture proteins was homologous) groups. However, all mixture groups showed significantly higher % inhibition than single groups in heterologous combinations. While the 5-mixture group was significantly better than 4-mixture groups in terms of GIA activities to heterologous parasites, there was no difference between the 5 and 6-mixtures. These data indicate that a limited number of allelic combinations may cover the whole genetic population. In addition, using the GIA data, we mathematically identified 14 amino acid polymorphic sites which significantly impact GIA activities and estimated the best combinations of AMA1 vaccine which cover the 14 sites. This study strongly supports future AMA1 vaccine development.

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EVALUATION OF THE IMMUNOGENICITY AND VACCINE POTENTIAL OF RECOMBINANT *PLASMODIUM FALCIPARUM* MEROZOITE SURFACE PROTEIN 8

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The C-terminal 19 kDa domain of merozoite surface protein 1 ($MSP1_{19}$) is the target of protective antibodies but alone is poorly immunogenic. Previously, using the *Plasmodium yoelii* murine model, we fused *Py*$MSP1_{19}$ with full-length *P. yoelii* merozoite surface protein 8 (MSP8). Upon immunization, the MSP8-restricted T cell response provided help for production of high and sustained levels of protective *Py*$MSP1_{19}$ and *Py*MSP8 specific antibodies. Here, we assessed the vaccine potential of MSP8 of the human malaria parasite, *Plasmodium falciparum*. Distinct from *Py*MSP8, *Pf*MSP8 contains an N-terminal asparagine and aspartic acid (Asn/Asp)-rich domain whose function is unknown. Comparative analysis of recombinant full-length *Pf*MSP8 and a truncated version devoid of the Asn/Asp-rich domain, *Pf*MSP8 (ΔAsn/Asp), showed that both proteins were immunogenic for T cells and B cells. All T cell epitopes utilized mapped within r*Pf*MSP8 (ΔAsn/Asp). The dominant B cell epitopes were conformational and common to both r*Pf*MSP8 and r*Pf*MSP8 (ΔAsn/Asp). Analysis of native *Pf*MSP8 expression revealed that *Pf*MSP8 is present intracellularly in late schizonts and merozoites. Following invasion, *Pf*MSP8 is found distributed on the surface of ring and trophozoite stage parasites. Consistent with a low and/or transient expression of *Pf*MSP8 on the surface of merozoites, *Pf*MSP8-specific rabbit IgG did not inhibit the *in vitro* growth of *P. falciparum* blood-stage parasites. These studies suggest that the further development of *Pf*MSP8 as a malaria vaccine component should focus on the use of *Pf*MSP8 (ΔAsn/Asp) and its conserved, immunogenic T cell epitopes as a fusion partner for protective domains of poor immunogens including *Pf*$MSP1_{19}$.

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INFECTION-TREATMENT-VACCINATION TO PREVENT *PLASMODIUM FALCIPARUM* MALARIA INFECTION

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Sterilizing immunity against malaria infection is an important model for malaria vaccine development. We designed an Infection-Treatment-Vaccination (ITV) regimen as an experimental tool to evaluate whether sterile protective immunity to *Plasmodium falciparum* can be induced by wild-type (non-attenuated) sporozoite immunizations. We hypothesized that parasite exposure might be limited to sporozoite or early liver-stage with the administration of primaquine (PQ) in conjunction with weekly suppressive chloroquine (CQ) prophylaxis and a low parasite inoculum (total of 36-45 infectious bites). A total of 36 healthy, malaria-naïve adult subjects were enrolled in the study. Six subjects enrolled in the Pilot Phase of the study to assess the complete prevention of blood-stage parasitemia by PQ administered two days versus three days after a single ITV infection. Five of six of the subjects in the Pilot Phase of the CQ/PQ ITV remained blood smear negative, and quantitative RT-PCR results provided evidence that earlier administration of PQ may enhance its liver stage killing activity. An additional 24 subjects were enrolled in the Main Phase of the study, including 13 subjects who received CQ/PQ ITV with PQ given 1 day after sporozoite exposure, 6 subjects who received CQ/PQ but uninfected mosquito bites, and 5 subjects who received CQ ITV with PQ placebo given 1 day after sporozoite exposure. All Main Phase subjects who completed the ITV Phase of the study remained blood smear negative throughout the ITV Phase. Quantitative RT-PCR indicated that CQ/PQ has a variable impact on the presence of blood stage parasitemia, with parasite exposure in some subjects completely limited to the sporozoite or early liver-stage. Five weeks after the last dose of CQ, protection will be assessed in both the Pilot and Main Phase subjects by homologous challenge with five infectious mosquito bites. Six additional subjects will join the study at challenge serving as infectivity controls. Data will be presented on the ITV phase and the outcome of the homologous malaria challenge. We will also present our efforts to identify and assess antigenic targets that are preferentially induced in protected individuals.

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CHARACTERIZING EPITOPE HAPLOTYPE DIVERSITY IN PATIENT SAMPLES FROM THE MOZAMBIQUE PHASE IIB RTS,S/AS02 TRIAL

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The RTS,S malaria vaccine, which is undergoing a Phase III trial, is based on the highly polymorphic circumsporozoite protein (CSP). Genetic analyses of infections from previous trials have not identified differences between RTS,S and control vaccinees in parasite strains defined based on individual polymorphisms of the CSP, decreasing concerns that widespread vaccination could select resistant strains. However, these studies were based on Sanger sequencing of PCR amplicons spanning the csp gene. This approach does not allow characterizing all strains in an infection, since minority alleles in multiclone infections may be overlooked. Additionally, this approach enables analyzes of strains based on unlinked polymorphisms rather than on epitope haplotype-level. Epitope haplotypes are relevant because immune cells recognize the antigen coded by the specific combination of alleles in these strands. Thus, vaccine-induced responses selecting parasites should produce effects detectable at the epitope haplotype-level. We used PCR-based 454 next generation sequencing (NGS) to re-analyze 205 patient samples from a Mozambique Phase IIb RTS,S/AS02A trial. NGS data is challenging due to a high fraction of error in allele calls. We validated our approach through the study of samples with known mixtures of 1-9 parasite strains/sample. We compared several methods to correct for miscalls when studying haplotypes based on the whole csp amplicon (330 bp) and on the Th2R and Th3R epitopes. The best methods detected greater than 97% of true haplotypes of the validation samples and minimized the number of false positives. We implemented those approaches on the Phase IIb trial samples and confirmed the differences in multiplicity of infection between vaccination groups. Interestingly, even in patients with multiple infecting genotypes, there is a dominant genotype, perhaps indicating either differences in fitness or transmission. We confirm that 454 NGS data, after error correction, can produce valid data in single and mixed infections. We compare data produced by 454 NGS with other NGS technologies, i.e., Iron Torrent and MiSeq.

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FATTY ACID ELONGASE-DEFICIENT HUMAN MALARIA PARASITES ARE ATTENUATED IN LIVER STAGE DEVELOPMENT

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In malaria parasites, fatty acids are synthesized in the apicoplast organelle through type II fatty acid synthesis (FAS-II), a process that is important for liver stage development. In addition to FAS-II, the endoplasmic reticulum (ER)-localized long chain fatty acid synthesis (LCFA) elongase pathway is thought to participate in meeting fatty acid requirements by extending shorter chain fatty acids, which typically are the FAS-II end products, to long fatty acyl chains. The enzymatic steps of both pathways follow similar reactions. In *Plasmodium falciparum* (Pf), the first step of LCFA seems to be handled by three different condensation enzymes (beta-ketoacyl CoA synthases), which are likely to differ in their preferred substrate acyl chain length. We have also identified a putative reductase, a dehydratase and a *trans*-2-enoyl CoA reductase in the Pf genome. Our co-localization studies demonstrate ER localization of at least one of the condensation enzymes as well as the *trans*-2 enoyl CoA reductase. We have also investigated the role of the elongase condensation enzyme PFA0455c in LCFA. Our genetic deletion studies in Pf parasites show that this putative enzyme is not important for asexual blood stage parasite growth or sexual stage parasite development in mosquitoes. However Pf sporozoites lacking PFA0455c are severely attenuated in their growth and development in cultured HC-04 human hepatocytes, as they fail to stain with antibodies specific to the late liver-stage surface proteins merozoite surface protein-1 and erythrocyte binding protein-175. The sequential accumulation of knockouts of essential genes is an alternative to developing radiation-attenuated sporozoites as candidate prophylactic vaccines against malaria.

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A NOVEL APPROACH FOR GENERATION OF "FULLY HUMAN" THERAPEUTIC MONOCLONAL ANTIBODIES

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Fully human monoclonal antibodies (mAb) are envisioned as a new therapeutic approach for neutralization of infectious agents/toxins, while devoid of side effects associated to the use of mouse, humanized, or chimeric antibodies. The current challenge for generation of fully human mAbs is the low frequency of specific B cells in human blood, since antibody-secreting plasma cells reside in lymphoid organs and bone marrow. Approaches that use EBV-transformed human B cells impose difficulties for further development into clinical use, as EBV is a relevant human pathogen. We have generated humanized mice expressing HLA-DR4 molecules in a NOD.RagKO.IL2RgcKO background (DRAG mice). Upon infusion of HLA-DR-matched human hematopoietic stem cells the DRAG mice develop functional human B cells that secrete specific IgG antibodies upon vaccination with tetanus toxoid (PLoS One 6:e19826, 2011). Herein we show that the DRAG mice immunized with *Plasmodium falciparum* sporozoites *or* infected red blood cells elicit high titers of specific antibodies. We have tested three human myeloma cell lines that do not secrete immunoglobulins and are HAT-sensitive, namely K6H6/B5, SHM-D33, and HuNS1 (ATCC) for efficiency of cell fusion using B cells from human PBMCs. We found that K6H6/B5 cells were more efficient for generating human B cell hybridomas than SHM-D33 and HuNS1. As DRAG mice develop human B cells and respond to vaccination, providing a convenient source of human antibody-secreting plasma cells, ongoing experiments are now underway to generate fully human therapeutic mAbs using *P. falciparum*-immunized DRAG mice.

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ELECTRIC NETS AND STICKY MATERIALS FOR STUDYING THE OVIPOSITION BEHAVIOUR OF GRAVID MALARIA VECTORS

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Indoor malaria vectors sampling has become difficult due to continued insecticide use inside houses through long-lasting insecticidal nets (LLINs) and indoor residual spaying (IRS). Development of tools for studying oviposition behaviour of *Anophels gambiae*, the major malaria vectors, is important to target them properly outdoors at breeding habitats. The study was conducted under semi-field condition using insectary - reared gravid *An. gambiae* s.s. Methods of using electric nets (e-nets) and various sticky materials were developed for recollecting mosquitoes at artificial breeding sites in greenhouses. An electric net operated at 50% spark energy setting was more effective than the one operated at 100% spark energy setting (Odds Ratio, OR 0.46; 95% CI 0.39 - 0.53, $p < 0.001$). A ring of four e-nets electrocuted a good number of mosquitoes though no attractant was presented in the artificial pond (52.5, 95% CI 44.4 - 62.1). Most mosquitoes fell in first row of a collection device, yellow sticky board (OR 0.34; 95% CI 0.24-0.48, $p < 0.001$) which was not attractive to the mosquitoes by itself (OR 0.02, 95% CI 0.01 - 0.05, $p < 0.001$). Most mosquitoes were collected on the surface of the water (103.3, 95% CI 93 - 114.8) detergent being the most effective of the substances used (OR 2.85, 95% CI 2.02 - 4.01, $p < 0.001$). E-net settings and sticky materials were successfully modified for studying the oviposition behaviour of gravid malaria vectors. Furthermore, it was found that *An. gambiae* do land on water surface for oviposition.

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THE EFFECTS OF THAI HERBAL ESSENTIAL OILS ON THE OVIPOSITION-DETERRENT AND OVICIDAL ACTIVITIES OF *AEDES AEGYPTI* (LINN), *ANOPHELES DIRUS* (PEYTON AND HARRISON) AND *CULEX QUINQUEFASCIATUS* (SAY)

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The effect of oviposition-deterrent and ovicidal of seven essential oils were evaluated towards three mosquito vectors, *Aedes aegypti*, *Anopheles dirus* and *Culex quinquefasciatus*. The oviposition activity index (OAI) values of six essential oils namely *Cananga odorata*, *Cymbopogon citratus*, *Cymbopogon nardus*, *Eucalyptus citriodora*, *Ocimum basilicum* and *Syzygium aromaticum* indicated that there were more deterrent than the control whereas *Citrus sinensis* oil acted as oviposition attractant. At higher concentration (10%) of *Ca. odorata* (ylang ylang flowers) it showed a high percentage of effective repellency (ER) against oviposition at 99.4% to *Ae. aegypti*, 97.1% to *An. dirus* and 100% to *Cx. quinquefasciatus*, respectively. The results showed that the mean number of eggs were lower in treated than in untreated water. In addition, there was an inverse relationship between essential oil concentrations and ovicidal activity. As the concentration of essential oil increased from 1%, 5% and up to 10% conc., the hatching rate decreased. The essential oil of *Ca. odorata* at 10% conc. gave a minimum egg hatch rate of 10.4% (for *Ae. aegypti*), 0.8% (for *An. dirus*) and 1.1% (for *Cx. quinquefasciatus*) respectively. These results clearly revealed that the Thai essential oil of *Ca. odorata* served as a potential oviposition-deterrent and ovicidal against *Ae. aegypti*, *An. dirus* and *Cx. quinquefasciatus.*

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INSECTICIDE RESISTANCE MONITORING OF FIELD-COLLECTED *ANOPHELES GAMBIAE* S.L. POPULATIONS FROM JINJA, EASTERN UGANDA

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Development of insecticide resistance in the malaria vector *Anopheles gambiae* s.l. threatens the success of control programmes necessitating regular resistance monitoring to enable effective insecticide-based control. We assessed the resistance status of male and female field-collected *An. gambiae* s.l. from Jinja (Uganda) using WHO diagnostic concentrations of deltamethrin, permethrin, bendiocarb, fenitrothion and DDT. The contribution of cytochrome P450 enzymes to the resistance phenotype was evaluated using synergist bioassays. Samples were screened for the 1014S and 1014F kdr alleles and the genetic association between kdr and resistance tested. Only *An. gambiae* s.s. and *An. arabiensis* (≈70%) were present in these collections. Both species were fully susceptible to bendiocarb and fenitothrion. The LT_{50} (time of insecticide exposure required for 50% mortality) for permethrin was found to be two- and three-fold higher than the 1hr recommended WHO diagnostic exposure for resistance in female and male *An.gambiae* s.s. respectively, and over 5-fold higher for deltamethrin. Resistance was also detected to DDT in *An. gambiae* s.s. *An. arabiensis* were resistant to permethrin and exhibited reduced susceptibility to deltamethrin in females but were fully susceptible to DDT. The kdr mutation 1014S is now approaching fixation in *An. gambiae* s.s. from Jinja (≈ 95%) but at low frequency in *An. arabiensis* (0.07%). Despite the high frequency in *An. gambiae* a significant association between 1014S and resistance phenotype was found for permethrin ($p = 0.0399$) and deltamethrin ($p = 0.0354$). The kdr

mutation 1014F was also found at low frequency (0.33%) from a single 1014S/1014F *An. gambiae* heterozygote. Bioassays with the synergist PBO resulted in partial recovery of susceptibility to both insecticides suggesting the additional involvement of CYP450s in resistance. No effect of PBO exposure on DDT resistance was detected. A small number (0.22%) of *An. gambiae* s.s./*An. arabiensis* hybrids were found, suggesting gene flow between the two species may be occurring hence there is a possibility of introgression of resistance alleles between species. In this study, we observed that resistance in this population involves both target site (kdr) and metabolic mechanisms. The high levels of insecticide resistance encountered in the Jinja-mosquito population threaten vector control efforts.

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USING CATTLE TO AUTODESSIMINATE INSECT GROWTH REGULATOR, PYRIPROXYFEN TO MOSQUITOES BREEDING HABITATS BY *ANOPHELES ARABIENSIS*

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Larviciding could complement other malaria control programs that target adult mosquitoes. However, locating all mosquito breeding habitats and the high implementation cost related to this remain major challenges for this strategy. The autodissemination of larvicides by mosquitoes offer a new possibility for larviciding. Therefore, this study aims at assessing the possibility to contaminate *Anopheles* arabiensis while they feed on cattle treated with pyriproxyfen (PPF) and their potential to transfer PPF into their breeding habitats. The study was carried out in rural Tanzania. Two screen houses (SFS) were built and inside each a mud hut was built where a cow was introduced. Unfed adult female An. arabiensis were released inside the SFS to feed on the cows. Clay pots were provided as resting sites for blood fed mosquitoes. In the control SFS, a cow was brushed with corn oil only whereas in the treated SFS it was brushed with corn oil and treated with pulverized PPF (Sumilarv 10%). Temporary breeding habitats for mosquitoes were installed inside the SFS. Eggs and larval presence and emergence inhibition were monitored daily from two days after mosquito release. Approximately all released mosquitoes blood-fed successfully in both control and treatment. Majority of mosquitoes were found resting inside the clay pots, walls and roof of cattle shed indicating that these resting sites can be used to contaminate mosquitoes. Significant adult emergence inhibition was demonstrated in larval bioassays with treated SFS mosquitoes, proving that mosquito were able to pick up PPF. The study is ongoing, assessing whether the contaminated mosquitoes can retain and transfer sufficient dose of PPF to their breeding habitats and inhibit mosquito emergence. Successful autodissemination of PPF and significant adult emergence inhibition in the contaminated breeding habitats will confirm demonstrate the efficacy of this technique for controlling malaria vectors.

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SPECIES SHIFT IN *ANOPHELES GAMBIAE* COMPLEX: DO LONG-LASTING INSECTICIDE TREATED 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 malaria vector species ratio have coincided with the scaleup suggesting that some species are being controlled by ITNs/LLINs better than others. 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 was in Lower Moshi Rice Irrigation Zone, an area where *Anopheles arabiensis* predominates, and the second was in coastal Muheza, where *An. gambiae* and *An. funestus* predominate. Five pyrethroids and one carbamate insecticide were evaluated on nets in terms of insecticide-induced mortality, blood-feeding inhibition and exiting rates. In the experimental hut trials mortality of *An. arabiensis* was consistently lower than that of *An. gambiae* and *An. funestus*. The mortality rates in trials with pyrethroid-treated 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 blood-feeding inhibition (percentage reduction in biting rates) being consistent between species. Veranda exiting rates did not differ between species. Percentage mortality of mosquitoes tested in cone bioassays on netting was similar for *An. gambiae* and *An. arabiensis*. 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 scale up of LLINs. With continued expansion of LLIN coverage in Africa An. arabiensis is likely to remain responsible for residual malaria transmission, and species shifts might be reported over larger areas. Supplementary control measures to LLINs may be necessary to control this vector species.

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WESTERN VERSUS EASTERN AFRICAN EXPERIMENTAL HUTS FOR THE EVALUATION OF PRODUCTS: A STRENGTH, WEAKNESS, OPPORTUNITY AND THREAT (SWOT) ANALYSIS FROM COMPARATIVE TEST OF REPELLENTS AND INSECTICIDAL PRODUCTS IN BENIN

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Western and Eastern experimental huts are used to assess efficacies of house-hold mosquito control intervention such as long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS). Until recently, there is no report indicating the suitability of those experimental huts in the evaluation of repellents and insecticidal products. Thus, this study aimed at investigating on the potential use of Western and/or Eastern African experimental huts to assess vapour-phase repellent and insecticidal product by highlighting the Strength, the Weakness, the Opportunity and the Threat (SWOT) related to both type of huts. The evaluation of insecticidal product (OlysetNet) and vapour-phase repellents (Metofluthrin 0.00625% and Metofluthrin 0.0097%) was performed in both Western (verandah trap hut) and Eastern African experimental huts at Donoukin field station (Benin) in order to compare the efficacy of those products in both huts style. The blood feeding inhibition was similar in both types of huts ($P > 0.05$) however the corrected mortality showed a significant difference whether the ITN / repellent were tested in the western or eastern hut. The corrected mortality was higher in the eastern hut. The exophily and the deterrency effects of the ITN were higher in the "verandah trap hut" (67% & 9% respectively) than what was recorded in the east one (24% & 0% respectively). The "verandah trap hut" was found to be a good experimental hut in the assessment of deterrency effect rather than evaluating exophily effect regarding to vapour-phase repellent. Still for repellent, the east experimental hut was well fitted to measure the exophily effect but not the deterreny one. Our results emphasize the suitability of the eastern experimental hut in the evaluation of insecticidal product as this type of hut allow the best measure of key property of that product such as mortality and blood feeding inhibition. The eastern hut is also appropriate to assess the efficacy of repellent though the "verandah trap hut" was found to be suitable for the evaluation of the deterrency effect of the repellent.

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THE OCCURRENCE OF PHENOTYPIC INSECTICIDE RESISTANCE TO MAIN MALARIA VECTORS IN TANZANIA

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The development of insecticide resistance has compromised mosquito control efforts in many parts of the world. With increasing coverage of malaria vector control interventions such as Insecticide Treated Nets (ITNs) and Indoor Residual Spraying (IRS) in Tanzania, periodic monitoring of susceptibility status of malaria vectors to insecticides is essential to ensure early detection and containment of resistance. This paper reports the findings of the national survey carried-out in 2011 to monitor the susceptibility of malaria vectors to insecticides namely pyrethroids, organophosphates, carbamates and DDT. The survey was conducted in 14 sentinel districts distributed across Tanzania mainland. The standard WHO methods was used to detect knockdown effect and mortality in the wild female anopheles mosquitoes collected from sentinel districts. The WHO diagnostic doses of 0.05% Deltamethrin, 0.05% Lambdacyhalothrin, 0.75% Permethrin, 0.1% Propoxur, 1% Fenitrothion and 4% DDT were used. Our results showed that *Anopheles gambiae complex* were highly susceptible to propoxur and fenitrothion (mortality rate of 99-100%). They were however resistance to deltamethrin and permethrin (mortality rate less than 80%) in Muheza, Moshi and Arumeru. Similarly, resistance was registered to Lambdacyhalothrin in Moshi (mortality rate of 55% [95% CI, 51-59]) and Arumeru (mortality rate of 70%[95% CI, 62-78]). Marginal susceptibility to Lambdacyhalothrin was recorded in Muleba (mortality rate of 85% [95% CI, 76-91]) and Muheza (mortality rate of 82% [95% CI, 73-89]). Resistance to DDT was recorded in Magu (mortality rate of 80% [95% CI, 58-92]). The occurrence of pyrethroid resistance to the main malaria vectors in Tanzania is quite alarming especially at this time when pyrethroids are used in both ITNs and IRS. There is a need to employ a resistance management plan so as to prevent the spread of resistance and prolong the use of the current insecticides. Continued vigilant monitoring of malaria vectors for their susceptibility to insecticide is of paramount importance.

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IMPLEMENTING FULL COVERAGE OF LONG LASTING INSECTICIDAL NETS: A GOOD ALTERNATIVE STRATEGY AFTER CESSATION OR ABANDON OF INDOOR RESIDUAL SPRAYING

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From 2008 to 2010, the National Malaria Control Program (NMCP) implemented Indoor Residual Spraying (IRS) for the first time in the department of Oueme, Benin. This large scale campaign which has prevented more than 350,000 people from mosquito bites was highly successful with a drastic drop of 94% of the Entomological Inoculation Rate. Stopping IRS in the department of Oueme raised some public concern about the bounce of malaria transmission. Therefore, LLINs was given to every household at a rate of one bednet for two people. In addition, the impact of this full coverage in LLINs on malaria transmission was studied. This study was carried out in four districts of the department of Oueme previously under IRS. After cessation of IRS, Olyset nets were distributed with a rate of one bednet for 1.9 people apart from old bednets still in use in the households. Here we present entomological parameters monitored in the four districts during and after the cessation of IRS. In each district, 2 mosquito sampling points were randomly selected and 2 houses chosen per sampling point for mosquito collections to monitor malaria transmission. Adult mosquitoes were collected twice a month. All *Anopheles* mosquitoes caught were identified to species. Vector species were dissected to determine the age grading and the heads/thoraxes parts analysed by ELISA method to look for CSP antigens. Abdomens of females were used for PCR analyses to identify mosquito species and molecular forms of both An. gambiae. Sampling of mosquitoes using morning pyrethrum spray catches (PSC) and window exit trap was done to determine eventual changes in mosquito behaviour.Results obtained showed that the spontaneous and widespread use of LLINs is a strategy as effective as IRS. In fact, Anopheline aggressiveness was the same during both 2 periods (IRS and LLINs). Mosquito physiological rate did not increase after the replacement of IRS by LLINs. Instead, it dropped (OR=3.81 ; p<0.001). This leads to similar infectivity rates of An. gambiae for *Plasmodium falciparum* (CS+IRS = 0.02 ; CS+MILD = 0.032) (p=0.160). This is the same for the daily inoculation rate: EIR=13.11 infective bites for a period of 9 months under IRS and 11.48 after IRS cessation for the same period. The large-scale use of LLINs is an effective alternative to the cessation of IRS because of cost issues.

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EXAMINATION OF INSECTICIDE RESISTANCE IN *AEDES AEGYPTI* POPULATIONS IN NEW ORLEANS, LOUISIANA

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Aedes aegypti is the vector of some of the most prevalent arthropod-borne viral diseases in the world, including dengue virus (DENV) and chikungunya virus (CHIKV). Recent outbreaks of DENV in Florida and CHIKV in Italy highlight the ease of viral spread via infected travelers, and as *Ae. aegypti* is present throughout the southern United States, there is a significant public health concern that the viruses could become established in these regions. There are no currently available vaccines or treatments for DENV and CHIKV, making reduction of transmission through vector control programs the best hope for reducing the burden of disease. Monitoring of insecticide resistance in local mosquito populations is an important component of effective vector control programs. For the current study, we examined whether *Ae. aegypti* from New Orleans exhibited resistance to several common classes of insecticides, including Type 1 and Type 2 pyrethroids, carbamates, and organophosphates, using the CDC bottle bioassay method. A colony was established from *Ae. aegypti* eggs collected in New Orleans in July and August of 2011. *Ae. aegypti*

Rockefeller strain was used as a control colony of known susceptibility. Adult female mosquitoes were placed into glass bottles containing up to 10ug of each insecticide, and mortality was scored at 15-minute intervals for up to 60 minutes. Resistance was scored according to WHO guidelines, where mortality of >97% indicates susceptibility, 80-97% indicates possible resistance, and <80% indicates resistance. We found that *Ae. aegypti* in New Orleans are highly resistance to carbamate and Type 1 pyrethroid insecticides, while possible resistance was found to organophosphates. However, local *Ae. aegypti* were susceptible to Type 2 pyrethroids. These data indicate that varying levels of resistance is present in the New Orleans *Ae. aegypti* population. Continued monitoring of resistance levels, including in more focal populations throughout the city, will be used to inform local vector control efforts and outbreak response plans.

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CONTRIBUTION OF INSECTICIDE RESISTANCE TO THE PRESENCE OF MOSQUITOES RESTING ON ITNS IN BUNGOMA, WESTERN KENYA

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Insecticide treated nets (ITNs) are an important tool and have been observed to reduce morbidity and mortality in several places as well as reducing indoor vector density. Several reports have however shown increasing numbers of malaria cases and deaths in western Uganda despite high ITN coverage. Holes in ITNs can cause a net to fail in its ability to deter mosquitoes, however, the amount of holes needed to qualify a net as spoilt has not been characterised. This study will evaluate ITNs in a village in Bungoma, an area of high pyrethroid resistance and Gem, an area of mild pyrethroid resistance, for the presence of vectors. The ITNs will also be evaluated for the presence of anopheline mosquitoes resting on them. If found, the mosquitoes would be used to raise f1s for WHO susceptibility assays to compare their resistance status with those collected from other parts of the house. Results from this study will enable understanding of the levels of insecticide resistance that would protect malaria vectors from the lethal and repellent effects of ITNs.

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ENHANCED EFFICACY OF A LONG-LASTING INSECTICIDAL MOSQUITO NET, OLYSET® PLUS, INCORPORATING A MIXTURE OF PYRETHROID AND SYNERGIST AGAINST PYRETHROID-RESISTANT MOSQUITOES

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The increase in pyrethroid resistance in mosquitoes has become a serious threat for vector control, and new tools are urgently needed. While other applications such as Indoor Residual Spraying can use alternative insecticide classes, in the case of Long Lasting Insecticidal Nets (LLINs) there is no alternative to pyrethroids. The need to overcome one of the major mechanisms of resistance led to this study on the use of piperonyl butoxide (PBO) into LLIN fibers on all net surfaces. PBO has long been used as a synergist because it inhibits microsomal oxidases and esterases in insects. Both of these enzyme systems are enhanced in resistance insects to metabolize the pyrethroid and thereby negate the effect of the insecticide. Olyset® Plus is a newly developed long-lasting insecticidal mosquito net incorporating a mixture of 2% w/w permethrin and 1% w/w PBO on all sides of the net. WHO tunnel tests were performed to investigate the efficacy of Olyset® Plus against a resistant strain of *Culex quinquefasciatus*. Compared to an Olyset®Plus variant net (that was manufactured without PBO to provide a positive control) the Olyset® Plus induced higher blood-feeding inhibition in the resistant mosquitoes. These results indicate that PBO enhanced the efficacy of permethrin against this pyrethroid-resistant strain. In experimental hut studies, Olyset® Plus induced high levels of blood-feeding inhibition against pyrethroid-resistant strains of *Anopheles gambiae* s.s. In summary, Olyset® Plus was found to have improved efficacy against pyrethroid-resistant mosquitoes and is a promising new tool for the control of pyrethroid-resistant malaria-transmitting mosquito populations in Africa. Olyset® is a registered trademark of Sumitomo Chemical Company Limited.

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A PHYLOGENETIC AND FUNCTIONAL METAGENOMIC REFERENCE IN THE GUT ECOSYSTEM OF *ANOPHELES GAMBIAE*

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Host associated microbes are ubiquitous, yet our understanding of the interactive relationships is very limited. Mosquito gut represents an ecosystem that accommodates a dynamic microbiota. The genetic wealthy gut communities fundamentally affect various mosquito life traits, such as fecundity and immunity. We have described the taxonomic structure of gut microbial communities across mosquito life history using 16S rDNA tag in *Anopheles gambiae*. However, little is known about the genetic repertoire and functionality of the gut microbiota. In this study we conducted metagenomic DNA- and RNA-seq of adult mosquitoes. Using an assembly-driven approach, a 41.8 Mbp metagenomic reference was compiled from 5Gbp sequencing reads. The reference assembly was annotated taxonomically and functionally. KEEG based metabolic modules were reconstructed, which enables pathway guided community function analysis. A functional signature was exemplified by anti-oxidative switching before and after blood feeding. The bacterial genome of an isolate of *Elezabethkingia* sp. was sequenced. The mapping of metagenomic RNA-seq reads against the *Elizabethkingia* genome was used to demonstrate the behavior of an individual bacterium in the microbial community. The metagnomic reference provides a phylogenetic and functional resource for inference of functions in mosquito gut ecosystem, such as host-microbe interactions and evolutionary co-adaptation.

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DEVELOPMENT OF A NOVEL MULTI-PLEX PCR-ITS2 ASSAY FOR THE IDENTIFICATION OF *AEDES* MOSQUITO VECTORS IN THE SOUTH PACIFIC

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On the islands of the South Pacific mosquitoes from the subgenus *Stegomyia* serve as vectors for a wide variety of pathogens, both viral and parasitic. In the Samoan islands three members of this subgenus are present: *Aedes aegypti, Ae. polynesiensis*, and *Ae. upolensis*. The former two species play major roles in the transmission of dengue fever and lymphatic filariasis respectively, while the role of the latter species, *Ae. upolensis*, is less clear. Because these mosquitoes are confirmed or suspected vectors of disease it is critical that collected individuals be

identified correctly. Presently, identification of these species at the adult stage is based upon morphological differences. This can be problematic as older mosquitoes and specimens that are not well preserved can be difficult to identify. The development of an accurate and rapid method for the identification of these vectors is especially critical in the Samoan islands, where monitoring of mosquito populations for disease-causing pathogens is ongoing. Here we present a multi-plex PCR assay based upon amplification of the internal transcribed spacer (ITS2) in *Aedes* (*Stegomyia*). We collected mosquitoes from multiple locations on the Western coast of the island of Tutuila, American Samoa. We extracted DNA from single individuals representing each of the three species and sequenced the ITS2 region from six to nine individuals from each species. We performed multiple sequence alignments and identified insertion-deletions (INDELS) and single nucleotide polymorphisms (SNP). Species specific primers were designed based upon INDELS or SNPs using an internal mismatch primers (IMP) approach. We demonstrated primer specificity by performing PCR amplification of multiple individuals representing all three species using blind test samples. In addition we constructed a phylogenetic tree from ITS2 sequences of individuals collected in this study as well as from published ITS2 sequences from *Stegomyia* individuals collected throughout the South Pacific. Our data show that *Ae. polynesiensis* from Samoa show significant differences from populations collected by others previously in Fiji and French Polynesia.

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GENE EXPRESSION IN DENGUE-INFECTED *AEDES AEGYPTI*

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Aedes aegypti is the primary vector of dengue viruses world-wide. The absence of effective dengue vaccines and treatments, and environmental limitations on the use of chemical insecticides, create an urgent need for novel disease-control strategies. One alternative strategy for controlling dengue transmission includes genetic-based modifications of the vector to generate mosquitoes incapable of virus transmission. This strategy requires the identification of *cis*-regulatory elements to drive the expression of anti-dengue effector molecules in a tissue- and time-specific manner. Genes encoding transcripts accumulated highly following dengue infection in the midgut and/or salivary glands are ideal candidates for donating *cis*-regulatory elements. RNA-seq technology was used to assess changes in transcript accumulation during the course of DENV2 infection in the *Ae. aegypti* Chetumal strain, which is highly-susceptible to virus infection. We identified infection-associated changes in transcript accumulation in the midgut, the first mosquito organ in contact with the virus following an infectious blood meal, and the salivary glands, where the virus resides before being transferred to a new vertebrate host. These results allowed the categorization of *Ae. aegypti* genes/pathways affected by dengue infection. However, a comparative analysis of our data with those published previously supports a complex scenario in which transcriptional responses to dengue infection vary among mosquito strains and combinations of these strains and dengue virus genotypes.

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NEW INSIGHTS ON AN AREA OF SECONDARY CONTACT BETWEEN *ANOPHELES GAMBIAE* M AND S FORMS FROM POLYMORPHISM ANALYSIS OF INTRON-1 OF THE VOLTAGE-GATED SODIUM CHANNEL GENE

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In most of their range in west-Africa, *Anopheles gambiae* M and S molecular forms are strongly reproductively isolated and clearly identified based on SNPs in the IGS rDNA region, which co-segregate with a C/T substitution at position 702 of the intron-1 of the voltage-gated sodium channel gene. Previous data showed that 12 haplotypes are found in this intron, the most wide-spread ones being the two characterized by the mentioned C/T SNP. We here present the results of a novel survey of the intron-1 carried out on M and S populations across African and including areas of putative inter-form secondary contact at the western-most extreme of their range: 87 females from 12 African countries were identified based on PCR-RFLP and SINE-PCR methods and a 531 bp fragment of intron-1 was sequenced. Estimates of DNA polymorphism at intron-1 were obtained using DnaSP v. 5. TCS software was used to reconstruct a haplotype parsimony network. A total of 26 haplotypes were observed (Hd=0.830): alleles in populations from The Gambia and Guinea Bissau were grouped in 20 haplotypes (Hd=0.827), while most alleles from other regions were grouped in only 10 haplotypes (Hd=0.748). Average nucleotide diversity was also higher in Guinean (ϖ=0.54%) and Gambian (ϖ=0.56%) populations than in those from the rest of Africa (ϖ=0.24%). Statistics applied to detect departures from neutral expectations were positive in The Gambia (D=2.02) and Guinea Bissau (D=0.87), but negative (D= -1.55) in the rest of the range, indicating population structure in the westernmost area, possibly due to a decrease in population size and/or to balancing selection. All M-form individuals were C/C homozygotes at position 702 (with a single exception), but a C/T polymorphism was observed in some S-form populations. These results, the relationships among haplotypes and their geographical distribution will be discussed with particular reference to their contribution in shedding light to the unusual situation of a putative secondary contact zone between M and S forms in the western extreme of *A. gambiae* s.s. range.

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GENOME-WIDE EXPRESSION PATTERNS DURING DIAPAUSE INDUCTION IN *CULEX PIPIENS* MOSQUITOES

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Culex pipiens (L.), the northern house mosquito, is an important vector of several human pathogens including West Nile virus and filarial nematodes causing lymphatic filariasis. It is among the most geographically widespread mosquito species in temperate regions worldwide. Adult females are able to survive the adverse conditions associated with winter by entering diapause, a state of programmed developmental arrest. In *Cx. pipiens*, the environmental stimuli determining diapause are the lower temperatures and shorter photoperiods accompanying late summer or early fall. We performed comprehensive gene expression profiling in standard (25°C; 16h light/8h dark) and diapause-inducing (18°C; 8h light/16h dark) conditions at three time-points (8, 16, and 24 hours post-treatment) during the early pupal stage, an environmentally-sensitive period for diapause induction. Using ANOVA and the student's

t-test we identified 1131 differentially expressed genes (p-value ≤ 0.05 using Benjamini-Hochberg corrections). A gene network consisting of five modules was constructed based on correlated expression across the three time points. Genes in each of the five modules were used in pathway enrichment analysis in MetaCore (GeneGo Inc.). Network modules containing genes that were down-regulated in diapause-inducing conditions were significantly enriched for genes involved with glycolysis and gluconeogenesis, while modules that were up-regulated in diapause-inducing conditions were enriched for genes involved in transcriptional silencing and Notch signaling. This analysis offers novel insights into the molecular basis of diapause by identifying key networks of co-expressed genes.

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QUANTITATIVE TRAIT LOCI (QTL) OF BLOOD FEEDING TIME OF *CULEX PIPIENS*

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The daily cycle of blood feeding activity by mosquitoes is an important factor in the etiology of mosquito-borne disease transmission. For example, with lymphatic filariasis, the density of microfilaria in the peripheral blood of humans infected with periodic *Wuchereria bancrofti* coincides with the daily cycle of blood feeding activity of *Culex pipiens sensu latu*, the primary mosquito vector. Here we investigated the quantitative genetics of blood feeding activity among progeny from an F_1 intercross between *C. pipiens* (Shasta strain) and *C. quinquefasciatus* (Trinidad field isolate). The Shasta strain is a long-standing laboratory strain and females readily blood feed any time of day. Females from the Trinidad field isolate only blood feed after dark. To assess blood feeding preference, 5 to 7 day old female F_1 intercross progeny were provided access to an anesthetized rat for 30 min at 11:00 am and 8:30 pm during the same day. Day feeding mosquitoes were separated from those that later did feed at night later subjected to DNA extraction. Individual progeny were subsequently genotyped for a panel of microsatellite and SSCP markers developed from the *C. quinquefasciatus* genome sequence. Quantitative trait locus (QTL) analysis identified genome regions containing genes that influence day vs. night blood feeding behavior.

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SPECIES COMPOSITION INFLUENCES THE EFFECTIVENESS OF VECTOR CONTROL IN PAPUA NEW GUINEA

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Anopheles punctulatus and its sister species (*An. koliensis, An. farauti* 1, 2, and 4) comprise the *An. punctulatus* species complex (AP group) that are the primary vectors of both lymphatic filariasis (LF) and malaria in Papua New Guinea (PNG). Until recently, *Anopheles* species were partitioned via morphological distinguishable characters allowing resolution of only three different species. However, by incorporating data from genetic markers, we, as well as others, have identified 10 additional species in the AP group identifying some species incompetent for disease transmission. To control disease transmission by competent vectors, long-lasting insecticide treated bed-nets (LLINs) have been deployed across PNG. However, LLIN deployment does not take into account the different population demographics of AP sibling species, assuming a single strategy can reduce annual transmission potential (ATP). We hypothesize that regions with different AP species compositions will require a combination of vector control strategies to reduce ATP. We sequenced 50 *An. koliensis* and 50 *An. punctulatus* mosquitoes by restriction site associated DNA markers (RAD tags) from the Madang region in PNG. We found that *An. koliensis* had substantially more genetic diversity, with most diversity partitioned within populations. In contrast, An. punctulatus had less genetic diversity with most diversity partitioned among populations. We conclude that high gene flow among population of *An. koliensis* will limit local control efforts by creating a rescue affect, recolonization, via non-compliant villages. However, *An. punctulatus*, with seemingly isolated populations, could be effectively controlled at a local scale without worrying about rescue effects from non-compliant villages. By discerning the population demography in these two primary disease vectors, we have demonstrated the importance of considering the differing life histories of AP sibling species. This then suggests that it is necessity to establish specific LLIN protocols for regions containing different species compositions.

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GENOME-WIDE ANALYSIS OF GENES CONTROLLED BY JUVENILE HORMONE RECEPTOR METHOPRENE-TOLERANT IN THE DENGUE FEVER MOSQUITO, *AEDES AEGYPTI*

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Female mosquitoes utilize blood as a rich source of nutrients for their egg development, and as a consequence becoming vectors of numerous human diseases. A better understanding of mosquito reproduction will lead us to the discovery of novel mosquito control methods. Juvenile hormone (JH) plays a critical role in controlling gonadotrophic cycles of female mosquitoes by preparing tissues for blood digestion and egg development. JH deprivation in newly eclosed female mosquitoes results in impaired posteclosion (PE) development and blockage of egg maturation. The molecular mechanism of JH action is poorly understood. We conducted a detailed microarray transcriptome analysis of the female fat body (FB), a tissue crucial for reproduction. This analysis revealed two major gene clusters during PE development: early and late gene clusters, EGC and LGC, each containing over 1,000 genes. EGC transcripts were high at the beginning of the PE development declining to a background level by 72 h PE, while LGC transcripts had an opposite trend, attaining maximum at 72 h PE. JH titer reaches its peak at about 72 h PE suggesting that a high titer of JH inhibits EGC and activates LGC. Methoprene-tolerant (MET) is a putative JH receptor. We conducted a RNA interference screen combined with a transcriptomic analysis, which showed that in MET-depleted mosquitoes EGC transcripts were highly elevated, while LGC transcripts down-regulated. A link between MET and JH actions has been provided by the quantitative PCR analysis of selected EGC and LGC genes repertoire in JH-deprived PE female mosquitoes. Using a combination of bioinformatics and molecular tools, consensus MET-binding motif has been identified from a subset of MET down-regulated LGC gene sets and proved by gel shift experiments with *Aedes* and *Drosophila* nuclear extracts. Our results suggest a central role of MET in controlling metabolism and protein synthesis during JH-regulated PE development of female mosquitoes. This study provides an important insight to the understanding of the molecular basis of JH action in mosquitoes.

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ROLE OF ARTIFICIAL CONTAINERS AS BREEDING SITES FOR ANOPHELINE MOSQUITOES IN MALARIA HYPOENDEMIC AREAS OF RURAL BANDARBAN, BANGLADESH: EVIDENCE FORM A BASELINE SURVEY

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Within the framework of our Mapping Malaria Epidemiology of Bandarban project, a survey was conducted for breeding habitats of vector mosquitoes during September-October 2011. The survey was carried out in seven of the 24 study clusters in two unions (Kuhalong and Rajbila), based on malaria incidence. The survey team screened mosquito larvae from natural reservoirs irrespective to their size and artificial or natural containers where natural water (e.g. rain) can stand for few days following standard protocols. Twenty larval habitats from each selected cluster were surveyed. A total of 3,696 immature mosquitoes (larvae and/or pupae) representing five mosquito genera were recorded from 122 habitats. The collection was dominated by *Culex* spp. (n = 2082; 46 single and 28 mixed occupancy) followed by *Aedes* spp. (n = 1469; 36 single and 14 mixed occupancy). Immature stages of *Anopheles* species (n = 128) were collected from 25 habitats, nine of which were single occupancy whereas, 14 habitats were shared with *Culex* spp and the remaining two with *Aedes* species. Anopheline larvae were reared to adult before identification and seven species were recorded. Median temperature and PH of Anopheline larval habitat were 35°C (IQR: 32.5°C -36.0°C) and 7.4(IQR: 7.05-7.85) respectively. Rice fields have been implicated as the most preferred breeding site for *Anopheles* spp. Other Anopheline breeding sites include puddle, irrigation canal, animal hoof print, artificial container and livestock wallow. Among the collected anophelines, *An. kochi* and *An. vagus* were found in containers (abundant plastic buckets and cement tanks respectively). *An. vagus* is considered an important malaria vector in Bangladesh. However, the preferences of *An. vagus* to artificial container needs further attention and should consider an alarming sign to malaria control in Bangladesh.

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VIRUS, VECTOR AND HOST INTERACTION IN THE AMPLIFICATION AND TRANSMISSION OF RIFT VALLEY FEVER AND NDUMU VIRUSES DURING THE 2006-2007 EPIDEMIC/ EPIZOOTIC IN KENYA DESCRIBED THROUGH HOST BLOOD MEAL ANALYSIS

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Rift Valley fever is a zoonosis caused by RVFV, transmitted by mosquitoes. The maintenance of RVFV among vertebrates is unclear. Bloodfed mosquitoes collected during 2006/07 outbreak in Kenya were analyzed to understand the animals that amplified RVFV. Mosquitoes were identified to species & abdomens & heads separated, triturated & screened singly for cytopathy in Vero cells, followed by RT-PCR on positive cultures. Cytochrome b & *CO1* genes in extracted DNA were amplified by PCR & sequences of purified amplicons queried in GenBank & BOLD to identify bloodmeal sources. 773 samples - Garissa & 37 -Baringo were analysed. In Garissa, *Aedes. ochraceus* fed on goat(211, 38%) cattle(92,16%) donkey(60,11%) sheep(33, 6%) human(30,5%) camel(15, 3%) gazelle, L. Kuddu & bird. *Ae. mcintoshi* fed on goat(61,35%) cattle(27, 15%) donkey(22, 13%) sheep(11, 6%) human(9, 5%) & duiker(1). In Baringo, *Mn. uniformis* fed on sheep(9, 53%) cattle, goat, duiker, frog, rat while 2 *Mn. africana* fed on sheep. *Hodgesia* sp fed on human, cattle, sheep, goat, rat & frog. In Garissa 7 *Ae. ochraceus* & 2 *Ae. mcintoshi* were infected with RVFV. *Ae. ochraceus* that fed on human(1) goat(2) had bloodmeal infection & sheep (3) goat (1) had disseminated infection while *Ae. mcintoshi* that fed on donkey(1) goat(1) had disseminated & bloodmeal infection respectively. In Baringo *Mn. uniformis* that fed on sheep(4) goat(1) had disseminated & bloodmeal infection respectively. 1 *Hodgesia* sp with bloodmeal infection fed on human. In Garissa16 *Ae.ochraceus* & 5 *Ae. mcintoshi* were infected with Ndumu. Of these, *Ae.ochraceus* fed on goat(8), sheep(2), cattle while *Ae. mcintoshi* fed on goat(2) camel & donkey. *Ae. ochraceus* & *Ae. mcintoshi,* key RVFV vectors in Garissa, preferentially fed on goat while *Mn. uniformis* in Baringo preferred sheep. Sheep & goat were significant in amplifying RVFV in both areas. The observation that donkeys were hosts to RVFV vectors & possibly amplified RVFV is significant while the role of wild animals remains unclear. The data also suggest that RVFV & Ndumu were co-circulating during the outbreak.

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IDENTIFICATION OF NEW MOSQUITO ATTRACTANTS USING COMPOUNDS PRODUCED BY HUMAN SKIN BACTERIA

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The African mosquito *Anopheles gambiae sensu stricto* is highly competent for malaria parasites and preferably feeds on humans, which makes it one of the most effective vectors of the disease. Human body odours are presumably the most important cues that enable *An. gambiae* to find its host. The skin microbiota plays an important role in the production of human body odours and the human microbial and chemical signature displays a qualitative and quantitative correlation. We showed that skin bacteria isolated from the skin and grown *in vitro* on agar plates attracted *An. gambiae*. Semi-field experiments showed similar results and field experiments in Kenya suggested that skin bacterial volatiles also attract other disease vectors. Analysis of the volatiles produced by human skin microbiota grown *in vitro* led to the identification of 16 compounds, the majority of which had an effect on *An. gambiae* host-seeking behaviour. 3-Methyl-1-butanol enhanced the attractiveness of a synthetic blend by a factor of three, and could be used to increase mosquito trap catches for monitoring or vector control purposes. 2-Phenylethanol decreased mosquito catches of traps baited with a synthetic blend and may act as a spatial repellent. In order to examine the interaction between the microbiota on the skin and human attractiveness to mosquitoes, skin emanation and skin microbiota samples were taken from 48 individuals. The skin emanations from the individuals varied significantly in attractiveness to *An. gambiae* and several compounds originating from the skin were associated with individuals that were highly attractive or less attractive to mosquitoes. Individuals with a higher abundance of bacteria on their skin were more attractive to *An. gambiae*, whereas individuals with a higher diversity of skin microbiota were less attractive. Volatiles produced by the human skin microbiota play an important role in the host-seeking behaviour of *An. gambiae* and the abundance and composition of the skin microbiota determine an individual's attractiveness to mosquitoes. Optimised blends of the compounds identified can be used in push-pull strategies for the manipulation of mosquitoes, thereby reducing the number of malaria mosquitoes and the intensity of *Plasmodium* transmission.

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HUMAN IGG ANTIBODY RESPONSE TO NTERM-34KDA SALIVARY PEPTIDE AS BIOMARKER FOR EVALUATING EXPOSURE TO *AEDES AEGYPTI* BITES

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Much effort is being devoted for developing new indicators to evaluate the exposure to *Aedes* vector and the risk of arbovirus transmission. Human antibody (Ab) responses to mosquito salivary components could represent biomarker for evaluating the real man-vector contact. To develop biomarker of human exposure to *Aedes aegypti* bites, we followed IgG Ab level to *Ae. aegypti* Nterm-34kDa salivary peptide in exposed children in Benin. Specific IgG response presented high inter-individual heterogeneity between the studied villages. IgG response was associated with rainfall and IgG level increased from dry (low exposure) to rainy (high exposure) seasons. It suggests the potential of such biomarker to detect variation in vector density. This preliminary study highlights the potential use of Ab response to this salivary peptide for evaluating human exposure to *Ae. aegypti*. Such biomarker should be a new tool to survey the risk of arbovirus transmission and to evaluate the vector control efficacy.

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CAN OLFACTORY MEMORY ALTER OVIPOSITION CHOICE IN MALARIA VECTORS?

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Oviposition site selection in mosquitoes is mediated by physical and chemical factors present in the aquatic habitats. Various organic materials and bacteria have been associated with this process. Though mosquitoes are innately attracted to or repelled by certain compounds, the chemical character of potential oviposition sites change over space and time. Mosquitoes might respond to this variability by altering their behaviour based on prior experience as a larva. Olfactory memory and its impact on oviposition choice has been described for *Aedes* and *Culex* species but has not yet been explored in anophelines. If the oviposition behaviour is inherited and defined in *Anopheles* mosquitoes, this means that the chemicals involved in this process can be identified, manipulated and used in 'attract & kill' or 'push-pull' strategies to improve malaria vector control. To investigate olfactory memory in oviposition by *An. gambiae*, larvae were reared in grass infusion previously avoided by gravid adults in open field habitats. The resulting adults were offered grass infusion and tap water in two-choice cage bioassays. Preferences were compared to mosquitoes reared in tap water. Mosquitoes that were conditioned in grass infusion and those reared in tap water preferred to lay eggs in tap water. The repellent effect of the grass infusion cannot be altered by rearing the vector in it. In contrast to some *Culex* and *Aedes* species the ability of *An. gambiae* to select oviposition sites appears not to be affected by the experience of inmature stages.

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AEDES AEGYPTI LONGEVITY AND ITS IMPLICATIONS FOR CLIMATE CHANGE AND MOSQUITO-BORNE DISEASE

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The dengue vector, *Aedes aegypti*, is well established throughout urban areas of the Southwestern United States. However, local transmission of dengue virus in Tucson and Nogalas, AZ has yet to be reported despite active transmission occurring only 170 miles south in Sonora, Mexico. One possible explanation for this is that *Ae. aegypti*, the primary vector of dengue, experiences a shortened lifespan when living at the northern edge of its ecological range. Since a mosquito must survive the 10-14 day extrinsic incubation period of the virus before being capable of transmitting dengue, reductions in lifespan can have a large impact on disease transmission. Using modified protocols for age grading individual wild mosquitoes we assessed the age structure of *Ae. aegypti* populations in southeastern Arizona. Mosquitoes were grouped as either non-vectors (< 5 d), unlikely vectors (6-14 d), and potential vectors (> 15 d). The percentage of parous mosquitoes, or those mosquitoes that had completed at least one reproductive cycle, in Tucson ranged from 40% in 2009 and 2011 to 51% in 2010. In Nogales, parity ranged from 34% in 2010 to 44% in 2011. We estimated the age of individual mosquitoes using a model based on the expression of the age-associated gene SCP1. Based on this model only 2% of the mosquitoes collected in Tucson in 2010 and 10% in 2011 were >15 d. In Nogales, AZ, 12% of the collected mosquitoes in 2010 and 9% in 2011 were >15 d. Combined with the parity data these results suggest that the age-structure of *Ae. aegypti* mosquitoes in southeastern Arizona is quite young and might be one of the factors contributing to the lack of dengue transmission in southeastern Arizona.

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DISTRIBUTION BIOLOGY, GENETIC AND ECOLOGICAL STRUCTURE ACROSS SELECTED POPULATIONS OF THE MAJOR MALARIA VECTOR *ANOPHELES GAMBIAE* IN UGANDA

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Malaria is a disease that remains of public health concern worldwide. Despite control initiatives including insecticide residual spraying, use of insecticide treated nets, intermittent preventive therapy in pregnancy and artemisinin-based combination therapy in Uganda, the malaria burden still remains. The development of genetically modified mosquitoes (GMMs) is an attempt for malaria control. A GMM has been developed for the *Anopheles gambiae s.s.* mosquito, an important vector of malaria worldwide and a predominant vector in Uganda. In order to evaluate GMM effectiveness in Uganda, well characterized confined potential field sites need to be identified. This research will fill knowledge gaps in vector bionomics critical to embarking on or expanding a malaria control program. Objectives of the study are to determine *An. gambiae s.s* geographic and seasonal dynamics across Uganda in terms of: i) relative abundance (ii) genetic and ecological structuring. The study will be carried out at five selected locations with *An. gambiae* populations falling roughly along an East-West transect across the River Nile. Adult Anopheline mosquitoes will be collected both indoors and outdoors using aspirators and CDC light traps, from randomly selected houses for a period of ten months. Larvae and pupae will be collected from breeding habitats and sent to the insectary for rearing. Habitat types will be recorded. Adults will be identified morphologically and molecularly. The species number and abundance per sampling will be documented. Daily records of climatic conditions will be obtained from the Meteorological department.

Variations in abundance due to environmental conditions and the association between *An. gambiae s.s* and other Anopheline species will be computed. Confirmed *An. gambiae s.s* from the different areas will be genotyped to determine extent of genetic differentiation. This study will contribute to baseline entomological data that is required for the selection of potential sites for future field releases of GMMs in Uganda.

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A NOVEL APPROACH OF EVALUATING AND ANALYZING OVIPOSITION BIOASSAYS FOR MALARIA MOSQUITOES

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As physiological resistance to insecticides and behavioural avoidance of interventions continue to defy costly frontline mosquito management strategies in Africa, the need to develop and integrate sustainable and effective solutions for dealing with these errant vectors of malaria remains pressing. A good understanding of the process of host seeking in *Anopheles gambiae* s.l. has instigated many effective intervention strategies. In much the same way, new insight on the little defined return journey when gravid mosquitoes leave their resting places after successful blood meals and lay eggs in select aquatic breeding sites could inform new fronts in management of these vectors. We looked into the periodicity of oviposition together with egg number and distribution as well as the impact of different host blood meal sources with the object of developing sound systems for studying the process and cues of oviposition site selection in *An. gambiae* s.l. The choice of host is demonstrated to influence the proportions of caged *An. gambiae* s.s. that become gravid following blood meals in favour their natural host, humans. Individual mosquitoes showed a wide variability in numbers of eggs laid and spread these across more than one substrate making it difficult to quantify its preferences for oviposition. Caged gravid mosquitoes consistently laid eggs in early scotophase with over 90% laying eggs by 21:00hrs. We present a novel way of implementing and analysing data from oviposition bioassays for *An. gambiae* s.l.

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POPULATION STRUCTURE OF THE MALARIA VECTOR *ANOPHELES DARLINGI* ROOT IN COLOMBIA

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Anopheles darlingi is the most important malaria vector in South America, including Colombia, where its distribution is irregular and the Andes Mountains may represent an important barrier for dispersal. Population genetics studies on malaria vectors can provide information about gene flow patterns and population differentiation that may influence vector capacity and behavior. In Colombia, previous studies on western and northwestern *An. darlingi* populations indicated minimal population structure and little evidence that geographic separation influences genetic divergence between geographic populations; however, the number of sites analyzed was small and not representative of the entire distribution of this species in Colombia. Therefore, the objective of this work was to evaluate the population structure of *An. darlingi* from seven sites at various geographical locations in Colombia. A 1,180 bp fragment of the cytochrome oxidase subunit I (*COI*) was analyzed for 190 specimens. Molecular variance and F_{ST} values showed evidence of population structure between northwestern (NW) and southeastern (SE) populations, whereas levels of differentiation were low within the NW populations and moderate in the SE populations. Estimates of the number of migrants (N_m) were between 0.1 - ∞. Tests for neutral evolution resulted in negative and non significant values in most cases. Haplotype networks revealed two deeply divergence clades: I represented the NW and II, the SE. NW populations were genetically closer to Central American populations and SE to those in South America. Our results showed strong population structure that may be influenced by geographical barriers to gene flow, such as the Andes Mountains; furthermore, the divergence observed between the two clades may reflect different demographic histories.

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A COMBINED APPROACH BASED ON WING GEOMETRIC MORPHOMETRICS AND MOLECULAR ANALYSIS FOR DISCRIMINATING BETWEEN *ANOPHELES CALDERONI* AND *AN. PUNCTIMACULA*

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Anopheles punctimacula Dyar & Knab and *An. calderoni* Wilkerson (Diptera: Culicidae) are very closely related species which are considered potential malaria vectors in Colombia. Females of these two species are quite difficult to distinguish using traditional morphological characters; therefore, complementary techniques are needed. Given the importance of an integrative taxonomic approach, a combination of analyses was used to identify adult females of these species collected in seven northern and western Colombian localities and included standard morphology, geometric morphometrics (GM) of the left wing and genetic analysis of the *COI* barcode and ITS2 sequences. The *COI* sequences were matched with reference sequences in the BOLD (Barcode of Life Data Systems) and ITS2 were verified with those in GenBank databases. Each specimen was confirmed to the species level. In GM analysis, 13 wing landmarks were digitized on specimens identified as *An. punctimacula* (*n*=22) and *An. calderoni* (*n*=21). The morphometric results obtained in the Procrustes PCA of these landmarks-registered in the PC space suggested that wing shape differed significantly between species. Furthermore, in the morphometric discriminant function analysis, the correct attribution of taxa before and after validated classification with the correct *a priori* groups was about 80%.The molecular analysis with both ITS2 and *COI* barcode sequences identified two clusters corresponding to the two species. These results indicated that a diagnostic signal is present in wing shape suggesting that the application of GM analysis could be used as a complementary tool for species identification, an important first step in potential vector incrimination and targeted control.

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LONGITUDINAL EVALUATION OF CHANGE IN THEIR QUALITY CONTAINER FOR OVIPOSITION

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Understanding oviposition behavior is key to predicting mosquito population dynamics and designing vector control interventions that effectively prevent disease. Site selection by female mosquitoes is influenced by a number of factors, including the perceived suitability of larval development sites, habitat availability, and population density. Previously we documented that oviposition by *Aedes aegypti* mosquitoes, the vector of dengue virus, is positively influenced by the presence of conspecific larvae in water-holding containers. Based on follow up

experiments, we show that this behavior may depend on the density of adult mosquitoes. Over three separate trials conspecific attraction to occupied containers was strongest when adult population size was the lowest. At deposition rates of 5-8 eggs per container per day, nearly 2x (1.7) more eggs were laid in containers with conspecific larvae while at deposition rates between 57-93 per container per day we could detect no differences between containers. Rates of deposition did increase in time, however, consistent with increasing food availability in naturally exposed containers. Furthermore, deposition into control containers with clean water declined as organic matter accumulated in treatment containers, indicating increasing attraction to containers with more food. It may be that an innate preference for inhabited sites was washed out by the large number of ovipositing female mosquitoes in the follow up trials. Alternatively, site selection behavior could be conditional on the behaviors of other individuals. An implication of this result is that larval control efforts will require increasing effort as populations fall in order to achieve abundances that are not conducive to pathogen transmission.

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INTEGRATED CONTROL OF DENGUE VECTOR BY *MESOCYSLOPS* AND *BACILLUS THURINGIENSIS* FROM LAHORE, PAKISTAN

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The present study evaluated the predatory capacity and efficacy of a local strain of copepod *Mesocyclops leuckarti (M. leuckarti)* and a bacterial strain, *Bacillus thuringiensis israelensis (Bti)* for the control of *Aedes aegypti* larvae. The main objective was to develop a cost-effective and environment friendly integrated vector control model in Lahore, Pakistan. *M. leuckarti* was collected from an artificial pond in the Lahore zoo. Single species culture was established in laboratory. *Aedes aegypti* reared in laboratory were used to evaluate the toxic effect of *Bti*. Larval mortality was evaluated singly and both with *Bti* +copepod in the field using 4 litre containers for 10 weeks. *M. leuckarti* and *Bti* showed 100% larval mortality during the first week of field experiments when used singly, which declined to 94 and 64% in the following weeks up to the week 05 respectively. At the end of fifth week *Bti* was not effective to kill larvae and reapplication caused 80-91% mortality by the end of week 10. In an integrated group (*M. leuckarti* + *Bti*), larval mortality was 99.3% by the end of week 5. Reapplication of *Bti* in this group during sixth week caused 100% mortality which remained 99.6% by the end of week 10. Therefore, an integrated control was found to be an effective strategy for the control of dengue vector in Pakistan.

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LONG-TERM IMPACT OF COMBINED SEWER OVERFLOW (CSO) REMEDIATION ON WATER QUALITY, MOSQUITO ABUNDANCE AND WEST NILE VIRUS AMPLIFICATION

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Combined Sewer Overflows (CSO) are a common wastewater treatment practice in ~750 US cities and a major source of urban water pollution. In urban Atlanta, GA, *Culex quinquefasciatus* abundance and West Nile Virus (WNV) transmission were found to be significantly higher at and near CSO impaired streams, where organically rich sewer discharges coupled with large forested and residential areas resulted in optimal conditions for mosquito larvae and opportunities for virus transmission. To comply with federal regulations, the City of Atlanta (GA) initiated major CSO improvements with the goal of substantially reducing sewer overflows. Here we report results of a longitudinal (2008-2011) comparative study assessing the long-term impacts of CSO-facility remediation on water quality and *Cx. quinquefasciatus* abundance. We compared immature and adult numbers between a CSO-affected creek (Tanyard) and a non-CSO affected stream (Peavine) one year before and three years after Tanyard Creek facility remediation. A drastic and significant reduction in median mosquito larval counts at Tanyard Creek was observed following facility completion from 5.80 before to 0.10 after remediation. Water quality indicators followed the same trend as mosquito numbers, with nitrate being the only chemical not affected by facility remediation. Mosquito abundance and water quality remained constant over time in Peavine Creek. The best generalized estimating equation model included creek, rain and water temperature, each organic nutrient concentration, interaction of all nutrients (excluding nitrate) by time since facility change, dissolved oxygen by temperature and creek by time since facility change as the main predictors of immature mosquito abundance. Our study shows that the reduction in mosquito abundance and improvement in water quality were the result of the CSO facility remediation. Improvements in wastewater management practices across the US could play a significant role in mitigating negative health effects of CSOs, including the potential reduction of vector-borne illness such as WNV.

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EVALUATION OF FIELD FEEDING ASSAYS IN PREPARATION FOR TESTING TRANSMISSION BLOCKING VACCINES IN MALI

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Transmission blocking vaccine (TBV) is an integral part in malaria control and eradication. Methods to evaluate TBV efficacy is a critical element in TBV development. Feeding assays where laboratory-reared mosquitoes were fed with malaria-infected blood, together with vaccine-induced antibodies, have been used to evaluate efficacy of the vaccine to block parasite development in mosquito midgut. The current study aims to develop and standardize these methods in preparation of a Phase 1b trial in Malian Malaria endemic area testing a candidate TBV made with a surface protein Pfs25 of *Plasmodium falciparum* ookinete stage. Direct Skin Feeds (DSF), where mosquitoes were allowed to feed directly on volunteer's leg, and Direct Membrane Feeds (DMF), where mosquitoes were feed on blood collected from volunteers and place in an artificial membrane feeder, were conducted in Mali to establish experimental infection baseline prior to the vaccine trial. Volunteers were consented and recruited on site based on blood smears reading results for gametocytes and trophozoites. Lab-reared mosquitoes were tested and free of known transovarially transmissible rivuses. For DSFs, a total of 30-60 mosquitoes were used. Volunteers participating in the DSF were followed closely for any potential DSF-related AEs. For DMFs, multiple samples were set to test whole blood, washed whole blood with and without autologous plasma being replaced with naïve sera. About 8 days after the feeding, mosquitoes were dissected and oocysts count in midguts was measured under microscopy. DSF was safe because there was no feed-related AE in participants. The infectivity rate was higher with DSF compared to DMF method. Blood washing steps during plasma replacement reduced the infectivity in DMF; but replacing autologous plasma with a naïve sera pool from US volunteers restored infectivity in DMF. In conclusion, DSF is safe and better suited for evaluation of transmission blocking vaccine in malaria-endemic areas.

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COMPARATIVE EFFICACY OF EXISTING SURVEILLANCE TOOLS FOR *AEDES AEGYPTI* IN WESTERN KENYA

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Traditional surveillance techniques for *Aedes aegypti* have all been developed for the cosmopolitan, domestic subspecies, *Ae. aegypti aegypti*, and not the sylvatic subspecies, *Ae. aegypti formosus*. In Western Kenya, the predominant form is *Ae. aegypti formosus* and is rarely associated with human habitations or linked to human dengue transmission. In this study we compared five mosquito surveillance methods for effectiveness in sampling *Aedes aegypti formosus* with the goal of determining a sustainable surveillance strategy to support on-going and future surveillance efforts in Kenya. The surveys were carried out in the Kisumu and Kakamega districts of Western Kenya. Each location consisted of four blocks sampled during wet and dry seasons over the period of one year. Surveillance methods included: larval and pupal surveys, oviposition traps, BG-Sentinel traps baited with BG-Lure, resting boxes and backpack aspirations that were randomly rotated between the four blocks after each successive trapping period. *Aedes aegypti* represented 25.5% of the total number of mosquitoes collected (n=2089). Larval and pupal surveys collected the highest number of *Ae. aegypti* (51.3%), followed by oviposition traps (45.7%), BG-Sentinel traps (3.0%) and zero collected with either backpack aspiration or resting box collections. Thirty containers out of 42 found with immature mosquitoes contained *Ae. aegypti* larvae and pupae. No larvae or pupae were found indoor and outdoor pots and tree holes were the most preferred oviposition containers of *Ae. aegypti*, contributing a respective 23.8% and 16.7% of the total containers. The results of this study indicate that outdoor larval and pupal surveys and oviposition traps were better surveillance methods of *Ae. aegypt*i in Western Kenya compared to other adult surveillance methods. Despite evidence indicating the *formosus* subspecies is a less competent vector for dengue than *Ae. aegypti aegypti*, the number and frequency of dengue outbreaks in Africa are on the rise. The need for further research to develop efficient ways of trapping adult *Ae. aegypti formosus* mosquitoes and the apparent failure of the BG-Sentinel traps is discussed.

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LONGITUDINAL DISTRIBUTION STUDY OF THE MOLECULAR FORMS M AND S OF *ANOPHELES GAMBIAE* IN DIELMO, SENEGAL

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In sub-Sahara Africa, the *Anopheles gambiae* complex includes the major vectors of malaria. An. gambiae s.s. and *An. arabiensis* are the most important in terms of epidemiology. It has been shown that *An. gambiae* comprises two molecular forms, M and S. However, whatever the geographical region, it has been clearly demonstrated that the gene flow between M and S forms is very limited, revealing a current speciation phenomenon. We study the longitudinal dynamic of *An. gambiae* M and S molecular forms in a senegalese village, Dielmo in order to evaluate gene flow and to determine epidemiologic role of the two subspecies. Mosquitoes were collected monthly by human bait collection from January 2006 to December 2011. *Plasmodium falciparum* infections were detected by ELISA-CSP on heads and thoraces of anophelies. A leg or wing was used to identified sub-species and molecular form according to PCR-RFLP method. In Dielmo, insecticide-treated nets (ITNs) were offered to all villagers since July 2008. A total 14,292 *Anopheles* specimens were sampled during 744 man night captures, among them 62% were *An. gambiae* s.l. In this study we tested 1,494 *An. gambiae* s.l. for taxa identification. Among them 24,6% were classified as *An. arabiensis*, 25, 5% molecular form M, 49,7 % form S and only 0,2% MS hybrids. The number of MS hybrids obtained was significantly inferior in the case of panmictic crossbreeding. A significant difference in CSP rate was observed between subspecies (Fisher's p = 0.002) with more infected mosquitoes in form S, but after implantation of ITNs, CSP rate decrease in *An. arabiensis* and the form M. Our study show that the molecular forms M and S are sympatric in Dielmo with a low proportion of hybrids what proves that the process of speciation between these two forms is very advanced. Also the molecular form S is the major on malaria transmission.

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DISTRIBUTION OF *ANOPHELES GAMBIAE* S.L. IN DIFFERENT ECOLOGICAL ZONES OF TANZANIA: IMPLICATIONS FOR MALARIA VECTOR CONTROL

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Members of the *Anopheles gambiae* complex are important vectors of malaria in most parts of Tanzania. The species complexes exhibit an enormous diversity in their biology which impact greatly on their importance as vectors of malaria and their behaviour. Understanding the diversity of these vectors is crucial to developing sound and cost effective interventions for malaria control. This study investigated the distribution of *An. gambiae* complex in different ecological zones of Tanzania. The study was carried out in 13 districts located across various ecological zones of Tanzania. The major ecological zones in this study were coastal savannah, grassland savannah, forest and highlands. Wild anopheles mosquitoes were collected using indoor resting catch and exit traps. Mosquitoes were morphologically identified and thereafter, PCR-based standard methods were used to identify mosquitoes into their respective molecular levels. A total of 7,596 collected mosquitoes were morphologically identified as *An. gambiae s.l.* of which, 2,536 (33%) were subjected for PCR analysis. Out of 2,536 mosquitoes, 1,660 (70.8%) and 876 (30.2%) were identified as *An. arabiensis* and *An. gambiae s.s* respectively. Both species occurred in sympatry in 30.8% of the districts sampled; while *An. arabiensis* occurred alone in 69.2% of the study districts. *An. gambiae s.s.* predominated in the highland-forest districts while the *An. arabiensis* were almost in all ecological zones. There were no significant associations between high percentages of either *An. gambiae s.s.* or *An. arabiensis* locations and the prevalence of malaria. The distribution of these two most anthropophilic members of the *An. gambiae s.l.* and malaria in Tanzania appear to be distinct, driven by different ecological factors. The findings from this study are of great implication for malaria vector control strategies. The implications of these findings in the context of malaria control in Tanzania are discussed.

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MOLECULAR IDENTIFICATION OF BLOOD MEAL SOURCES OF MOSQUITOES FROM THE AMAZON BASIN OF PERÚ

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The transmission dynamics of many arboviruses in the Amazon Basin region are not yet fully elucidated, including vectors and natural reservoir hosts. Consequently, identification of blood meal sources in field-caught mosquitoes could yield important information for identifying potential arbovirus vertebrate hosts. In this study, we sought to identify blood meal sources in mosquitoes collected from areas endemic for

alphaviruses in the Peruvian Department of Loreto using molecular approaches. From January-March 2009, mosquitoes were collected in forest, peridomiciliary, and intradomiciliary areas in four villages of the provinces of Alto Amazonas and Datem del Marañon, using CDC light traps, human bait, and backpack aspirators, respectively. A total of 119 field-collected engorged mosquitoes were identified using dichotomous key, homogenized individually, and subjected to DNA extraction and PCR amplification using consensus primers targeting the *cytochrome b* gene of mammals and birds. The performance of the molecular assay was previously validated using DNA extracted from blood of known vertebrate species. PCR amplicons were obtained from 105 mosquitoes; sequencing and GenBank BLAST search analyses based on >90% sequence similarity putatively identified the amplified sequences. *Psorophora albigenu* (n = 61) fed on humans, cows, spiny rats, and smooth-billed anis and *Ps. cingulata* (n=3) fed on humans. *Culex* (*Melanoconion*) *vomerifer* (n=2) and *Cx. (Aedinus*) *amazonensis* (n=1) fed on spiny rats; *Cx.* (*Mel.*) *occossa* (n=1) fed on humans; *Cx.* (*Mel.*) *dunni* (n=1) fed on two-toed sloths; and *Cx.* (*Mel.*) *portesi* (n=1) fed on cats. *Ochlerotatus fulvus* (n=8) fed on humans and cows. *Oc. serratus* (n=15) fed on humans, dogs, and on common moorhen. *Mansonia humeralis* (n=4) fed on humans. *Anopheles oswaldoi* s.l. (n=5) and *An. benarrochi* (n=3*)* both fed on humans and *An. benarrochi* also fed on pigs. Our results demonstrated that *Psorophora albigenu, Cx* (*Mel*) spp*., Oc. serratus,* and *Oc. fulvus,* three mosquito species implicated as alphavirus vectors, fed on humans in the Peruvian Amazon basin.

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ECOLOGY AND COMPETENCE OF GALÁPAGOS *CULEX QUINQUEFASCIATUS* AS A POTENTIAL VECTOR OF WEST NILE VIRUS

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Culex quinquefasciatus, a major vector of West Nile virus [WNV] in southern USA, was first detected on the Galápagos Islands (Ecuador) in the 1980s, with subsequent evidence of further introductions. However, little is known of the ecology of this mosquito in Galápagos, or how this might influence its vectorial capacity to transmit WNV should the pathogen be introduced to the archipelago. Concern exists for the impact that WNV may have on endemic Galápagos species given their lack of prior exposure to flavivirus and potentially heightened susceptibility to WNV. It is thus important to consider potential vectors that would facilitate future transmission of WNV in Galápagos. Here we characterise the vector ecology of *Cx. quinquefasciatus* in Galápagos, describing its life-cycle stage durations, spatial distribution, temporal abundance and host-feeding behaviour. Water salinities above 5 ppt were demonstrated to hinder larval development, which we suggest could limit the vectors distribution around the Islands. Analysis of blood-meals from wild caught mosquitoes indicates contact with reptiles, birds and mammals. We also report further details of the WNV competency of Galápagos *Cx. quinquefasciatus*, including evidence for vertical transmission (MFIR 3.7/1000), a potential persistence mechanism for the virus on the Islands. An ID50 dose of 7.4 log10 PFU/mL WNV was required to infect 50% of Galápagos *Cx. quinquefasciatus* (not significantly different to a control group of USA *Cx. quinquefasciatus*). Together these details are useful for epidemiological assessment or to assist vector control by deciding the relative importance of candidate vectors on Galápagos.

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LABORATORY TRANSMISSION AND CHARACTERIZATION OF A NOVEL MICROSPORIDIAN PARASITE FROM THE INVASIVE ASIAN ROCK POOL MOSQUITO, *OCHLEROTATUS JAPONICUS*

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Ochlerotatus japonicus is an invasive mosquito from East Asia that was first detected in the northeastern US in 1998. It has rapidly spread throughout much of eastern North America where it is now firmly established, and has recently been found in Belgium, France, Switzerland and Germany. It is an aggressive human biter and has been incriminated in transmission of several important arboviruses including Japanese encephalitis and West Nile virus. Surveys of North American populations have yet to uncover any significant natural enemies. A novel microsporidian parasite, first discovered in 1980 from *Oc. japonicus* larvae collected from rock pools along the Okudake River, Oita Prefecture, Kyushu Region of Japan, has been re-isolated from the same habitat and evaluated for introduction and establishment in the US as a potential biological control agent. This microsporidium has been found infecting natural larval populations of both *Oc. japonicus* and *Oc. hatorii.* It invades larval fat body tissue and typically kills its host just prior to pupation. The microsporidium is unikaryotic throughout development, undergoes asexual reproduction forming multinucleated schizonts and lanceolate spores in groups of eight within a sporophorous vesicle. Mature spores possess a large bilaminar polaroplast with voluminous chambers anteriorly, an isofilar polar filament with 2-3 coils, large posterior vacuole, and thin unornamented exospore. Laboratory transmission studies have revealed that spores are directly infectious to mosquito larvae, unlike the majority of other mosquito-parasitic microsporidia that require obligatory development in an intermediate copepod host. Orally infected larvae develop benign infections and survive to adulthood where the microsporidium is vertically (transovum) transmitted by females. The resulting F1 progeny develop patent infections that lead to the production of infectious spores that are re-released into the aquatic environment with death of the larval host. Phylogenetic analysis of the small subunit rRNA gene sequence place this microsporidium as a distinct sister taxon within the clade of microsporidian parasites of mosquitoes. A new genus and species, *Takaokaspora nipponicus* is proposed.

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INCREASED CAPTURE RATES OF ADULT SPECIMENS OF *AEDES AEGYPTI, AE. MEDIOVITTATUS* AND *CULEX QUINQUEFASCIATUS* THROUGH MODIFICATION OF THE BG-SENTINEL TRAP

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The BG-Sentinel (BGS) trap was specifically designed to capture *Aedes aegypti* females. Field observations suggested that BGS traps did not efficiently capture females of *Ae. mediovittatus*, the Caribbean treehole mosquito, which is a potential vector of dengue viruses in Puerto Rico. We conducted experiments in a large outdoor mosquito cage to determine if capture rates of BGS traps were biased against *Ae. mediovittatus* in comparison with *Ae. aegypti*, and to improve trap efficacy. Several chemical (BG-Lure, CO2, octenol) and visual (trap size, color) attractants were investigated. Field tests followed the discovery that the capture rate for females of both mosquito species could be significantly improved by replacing the white outer cover of the BGS trap with a black one. The field results showed that black BGS traps with BG-Lure captured significantly more *Ae. aegypti* (*38%*), *Ae. mediovittatus* (*79%*), and *Culex*

quinquefasciatus (15%) than the white ones in a rural neighborhood in Puerto Rico. The modified traps were more sensitive in detecting the latter two species, captured more mosquito species, and had a smaller ratio *Ae. mediovittatus* to *Ae. aegypti* females. We also observed that these *Aedes* species positively co-occurred at field sites with a significantly greater frequency (31.2%) than expected from the data collected by the original traps (19.9%).

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GENDER DIFFERENCES IN TUBERCULOSIS (TB) NOTIFICATION AND ADHERENCE TO TREATMENT IN KHARTOUM STATE, SUDAN

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Case detection is the key factor in the treatment and elimination of tuberculosis (TB). The aim of this study was to inform whether and how the risk of TB varies for men and women, and how gender affects motivation and access to timely health care in Khartoum state. A retrospective register based study was conducted during the period from January to December 2006.Three hospitals and eight health centers were included in this study to determine the sex ratios, age groups, areas, type of treatment and follow-up of patients with pulmonary tuberculosis. In addition, we looked into seasonal variations. We found that the reported incidence of pulmonary TB was lower in women than men; the men to women ratio was 3:1. The most affected age group was the productive group (15-35 years). The notification rate was higher in women (1.2 %) than in men (0.9%) in the youngest age group (0-15 years old). The highest percentage of TB cases was found in winter (November-March) (39.5%) and May (11.6%). In conclusion, we found that men have access to TB health services more than women. However, women have better adherence and are more likely to complete a full course of treatment.

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DETECTION OF *MYCOBACTERIUM TUBERCULOSIS* IN PARAFFIN-EMBEDDED TISSUE SHOWING GRANULOMATOUS INFLAMMATION BY PCR

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Tuberculosis is still an increasing health problem worldwide, therefore, a rapid and reliable diagnosis of cases is essential to initiate correct treatment, avoid severe complications, and prevent transmission. Conventional microbiological methods may not be the best option for the diagnosis in formalin-fixed, paraffin-embedded tissue (FFPE), showing granulomatous inflammation consistent with TB. However, histopathologic features of chronic granulomatous inflammation can be found in various conditions and diseases other than TB. Therefore, the detection of *Mycobacterium tuberculosis* DNA in a FFPE tissue is used for the early diagnosis of TB cases in these specimens, where morphologic features are suggestive, but not confirmatory of TB. A total of 56 FFPE tissue specimens suspected to have TB, either by clinical assessment and/or histopathological investigation were obtained from the Pathology Department of King Abdulla University Hospital. All specimens were tested by a hemi-nested PCR for a specific fragment of the 16S-rRNA, which is present in all *Mycobacterium* species. Positive specimens by this assay were tested for the presence of IS6110 insertion sequence that is specific to the *M. tuberculosis* complex. Results of PCR assays were compared to the histopatholoy results, and analyzed by Chi-square test. Of 56 specimens, 42 were positive for *Mycobacterium* species by the hemi-nested PCR, 38 of these were positive for *M. tuberculosis* complex by the IS6110 insertion sequence PCR. TB diagnosis was confirmed by PCR in 38 (68%) patients compared to 7/56 (12.5%) that were positive by tissue acid-fast stain, and the other 4/42 (7%) specimens were considered as atypical mycobacterial infections. The 14/56 (25%) specimens that were negative in both PCR assays were considered as TB negative cases. In conclusion, PCR proved to be more sensitive in the detection of *M. tuberculosis* complex, therefore, it is recommended for the diagnosis of TB suspected cases, when the FFPE tissues showing granulomatous inflammation are the only material available, and the acid-fast stain is not helpful in demonstrating the AFB, and/or no concurrent tissues are cultured.

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GREEN SYNTHESIS OF RIFAMPICIN-LOADED SILVER-STARCH BIONANOCOMPOSITES FOR THE THERAPEUTIC TREATMENT OF TUBERCULOSIS

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Estimates reveal that, more than 9 million cases of tuberculosis (TB) occur globally, with Asia and Africa accounting for 85 %. Unless more effective and patient compliant anti TB medications are available at affordable prices, the annual number of TB deaths can be expected to increase. Submicron carrier systems for the delivery of antibiotics are gaining increasing interest. However, the use of chemical methods raises concern for environmental contaminations as the chemical procedures involved in the synthesis of nanomaterials generate a large amount of hazardous byproducts. Plant extracts such as starch are very cost effective and eco-friendly and thus can be an economic and efficient alternative for the large scale synthesis of nanoparticles. Starch from *Manihot esculenta* was isolated, purified and converted into its acetylated (ACS), Hydroxypropylated (HPCS) and Succinylated (SCS) derivatives. We report herein the binding of Rifampicin to starch protected silver nanoparticles and its application in enhanced oral delivery of rifampicin for the therapeutic treatment of tuberculosis. NMR, FTIR and Raman spectroscopies were used to confirm the synthesis, while DSC-TGA, SEM, XRD, viscosity profile, water absorption and solubility indices were used to characterize the new polymer. Formation of Rifampicin-silver-starch bionanocomposites was confirmed by UV-visible spectroscopy, XRD, FTIR and SEM with Energy dispersive X-ray (EDX) patterns. Result of SEM shows that the bionanocomposites had a spongy appearance. The mean particle size for ACS, HPCS and SCS starches were 265, 273 nm and 6.736 mm respectively, PDI were 0.293, 0.302 and 0.592 for ACS, HPCS and SCS respectively, while the zeta potential values were -4.88, -20.07 and 13.69 mV for ACS, HPCS and SCS respectively. *In vitro* release of encapsulated nanoparticles show significant ($p < 0.05$) extended release profile up 12 h. *In vivo* anti tubercular screening shows that, the bionanocomposites and RIF-free composite exhibited MIC range between 0.001 - 0.003 and 0.06 - 0.08 mg/mL respectively. This study shows that, Rifampicin-loaded silver-starch bionanocomposites prepared from a cheap, non-toxic, renewable and generally compatible natural polymer could considerably improve rifampicin antibacterial efficacy, while still being more economical.

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COMPARISON OF PCR WITH STANDARD CULTURE OF FINE NEEDLE ASPIRATION SAMPLES IN THE DIAGNOSIS OF TUBERCULOSIS LYMPHADENITIS

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Lymphadenopathy is the commonest form of extrapulmonary tuberculosis (TB) Clinical diagnosis of TB in lymph nodes requires aspiration of the material and isolation of mycobacteria. Bacterial culture is the gold standard for detection of tubercle bacilli, but it is time-consuming and requires specialized safety procedures and a BSL3 laboratory. However, PCR is a rapid method which requires small volumes of samples and can also be performed on killed bacilli to ensure safety. This project was

designed to compare direct fine needle aspirate (FNA) PCR with culture in the diagnosis of tuberculosis lymphadenitis. Direct examination of samples with EZN staining, culture, cytology and PCR was performed on previously collected FNA from the patients with suspected tuberculosis lymphadenitis. In total, 38% of the samples were positive for TB by culture, 11.8% by EZN staining, 23.4% by PCR, and 59.8% by cytology. Cytology had the highest sensitivity (81%) and EZN stain the least (22.9%). The specificity of EZN stain was the highest (92.4%) while cytology was the lowest (50%). In this study, out of 50 culture-positive samples, 21 (42%) were positive by PCR while 8 (10.8%) out of 74 culture-negative samples were positive by PCR. Although PCR is a sensitive diagnostic method, its sensitivity was shown to be low in this study. Therefore, we recommend that further studies should be conducted on fresh aspirate samples to investigate for possible PCR inhibitors which may limit the sensitivity of PCR diagnosis.

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PREDICTORS OF MORTALITY AMONG TUBERCULOSIS AND HUMAN IMMUNODEFICIENCY VIRUS CO-INFECTED PERSONS IN SOUTHWEST ETHIOPIA: A CASE CONTROL STUDY

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Tuberculosis (TB) remains the most common cause of death in people living with HIV/AIDS. The aim of the present study was to identify predictors of mortality in TB-HIV co-infected patients taking anti-retroviral therapy (ART) in Southwest Ethiopia. We conducted an unmatched case control study among a cohort of TB-HIV co-infected adults who were on ART in the period June 08, 2003 to August 14, 2009. Cases were 69 TB-HIV co-infected patients who died during this period. For each case, we selected three (207) TB-HIV co-infected patients who were alive during the same period. Data were collected using a structured and pre-tested questionnaire. Bivariate and multivariate analysis was done to identify predictors of mortality using SPSS 16.0 statistical software. Of the 188 deaths registered in the study period, 69 (36.7%) were TB-HIV co-infected. Most (75.3%) of the deaths occurred during the first 6 months of initiation of ART. Male sex (OR=2.04, 95% Confidence Interval [CI]:1.04-4.02), being bedridden at enrolment (OR=2.84, 95%CI: 1.17-6.89), and cough of more than two weeks during initiation of ART (OR=4.75 95%CI: 2.14-10.56) were the best predictors of mortality among TB-HIV co-infected patients. In conclusion, mortality among TB-HIV co-infected patients accounted for a considerable number of deaths among the cohort. Patients with cough at ART initiation and with poor functional status should be strictly followed to reduce death.

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RATES AND CAUSES OF SERIOUS ADVERSE EVENTS IN THE FIRST 25 WEEKS OF A CLINICAL TRIAL OF MATERNAL INFLUENZA VACCINE DURING THE THIRD TRIMESTER IN BAMAKO, MALI

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Maternal and neonatal morbidity and mortality in West Africa are significant barriers to Millennium Development Goals 4 and 5. Maternal immunization trials in areas with limited health infrastructure present the opportunity for active surveillance of complications whose background rates are often poorly characterized. Here we present the rates and causes of maternal mortality, near-miss maternal morbidity, cesarean sections, stillbirths, neonatal mortality, and neonatal hospitalizations during the first 25 weeks of a trial of maternal influenza vaccine in the 3rd trimester in Bamako, Mali. Pregnant women are recruited from antenatal clinics during the 3rd trimester, vaccinated with influenza vaccine (Vaxigrip, Sanofi Pasteur) or quadrivalent meningococcal conjugate vaccine (Menactra, Sanofi Pasteur), and followed to 6 months after delivery. Follow up includes weekly home visits and attendance at all deliveries and participant hospitalizations. Detailed clinical reports are prepared on all events of interest and are reviewed by at least one obstetrician and pediatrician. Between September 12, 2011 and March 02, 2012, a total of 1,263 women were recruited, 725 of whom delivered. There were 725 live births and 6 stillbirths (2 fresh and 4 macerated). There was 1 maternal death (138 per 100,000 live births) from post-cesarean complications and 15 near-miss cases, of which 40% were hypertensive disorders and 33% hemorrhage. The cesarean rate was 6.6%. Over 17,835 days of observation during the neonatal period, there were 29 hospitalizations, most commonly due to complications of prematurity (45%) and neonatal infections (35%), and 5 neonatal deaths (6.9 per 1,000 live births). Maternal and neonatal complication rates during the first six months of this trial are substantially below published background rates from this region. While these results may in part be due to selection of healthier pregnancies among those receiving antenatal care, they confirm the positive impact on maternal and neonatal morbidity and mortality of better access to care during a clinical trial.

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NON-INFLUENZA ETIOLOGIES OF INFLUENZA LIKE ILLNESS IN PERU

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Respiratory viruses continue to be an important cause of morbidity and mortality worldwide. Recent advances in standardized molecular diagnostic assays have lead to the discovery of new viruses associated with respiratory infections, such as human coronaviruses (HCoV), bocaviruses (HBoV), and human metapneumovirus (hMPV). There is limited information on the epidemiology of these viruses in Peru. With the objective of describing the presence of non influenza respiratory viruses at the community level in Peru, we tested a subset of samples from a longitudinal multisite cohort study using a multiplex technology. Nasal and oropharyngeal swabs were collected from individuals presenting with influenza-like illness (ILI) in an active population-based cohort study in Peru. This project was initiated in June 2009, and consists of 6000 participants in 1500 households located in four ecologically distinct regions across Peru: Lima (central coast/urban), Tumbes (tropical coast/rural), Cusco (highlands/semi-rural), Puerto Maldonado (Amazon rainforest/urban). Samples were initially tested by rRT-PCR for influenza viruses A and B. A sub-group of 174 samples negative for influenza were selected for additional respiratory virus testing using the multiplex Luminex Respiratory Viral Panel Fast Assay. Viruses identified included enterovirus/rhinoviruses (33%), HCoV (10%), hMPV (6%),, parainfluenza (6%), adenovirus (2%), respiratory syncitial virus (2%), and HBoV (0.6%). Co-infections with at least two viruses were identified in 10 (6%) of the participants. We were unable to detect a virus from 67 (39%) of the

samples tested. These results demonstrate that a variety of respiratory viruses, including novel hMPV, HCoVs and HBoV can be found in patients with ILI in Peru. Viruses were identified in 61% of ILI samples negative for influenza. Although complete diagnosis of respiratory viruses associated with ILI is not sustainable due to high costs, limited etiological surveys may provide useful information on the burden of respiratory disease attributed to these agents in Peru.

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MOLECULAR CHARACTERIZATION AND DRUG SENSITIVITY TESTING OF *MYCOBACTERIUM TUBERCULOSIS* ISOLATES FROM RURAL PULMONARY TUBERCULOSIS PATIENTS IN EAST GO JAM, NORTH WEST ETHIOPIA: A PRELIMINARY REPORT

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A total of 364 sputum samples were collected cross-sectionaly from all consecutive pulmonary tuberculosis patients visiting the study sites in East Go jam, North West Ethiopia. One hundred fourteen (31%) were found to be culture positive. Region of difference analysis for RD9 (RD9 typing) indicated that 105 isolates were *Mycobacterium tuberculosis* species. Spoligotyping of 46 *M. tuberculosis* isolates showed that SIT 910 with 7 isolates and SIT 149 with 5 isolates were the predominant spoligotypes in the area. The spoligotypes were grouped into ten clusters which represent 78.2 (36/46) of all isolates typed. Nine spoligotypes, one with a cluster of five isolates and eight single strains, were never reported anywhere in the world and new to the international database, SpolDB4. The most prevalent lineage identified in this study was the Europe-American lineage 65% (13/20). Sensitivity testing was available for 63 of isolates. In total, 18(28.5%) of the isolates were resistant one or more of anti tuberculosis drugs isoniazid (9.5%), rifampicin (6.3%), ethambutol(9.5%) and streptomycin(3.2%) in which 26.7% were among new patients and 42.8% among previously treated patients . Three (4.7%) isolates (1 from the new and two from previously treated patients) were MDR TB showing that MDR TB is prevalent in the area. A large proportion of clustering of the isolates indicates a high rate of exogenous TB transmission in area. Prevalence of multi drug resistance among new cases was relatively similar with the previous reports in the other parts of the country but is higher among retreatment cases. Strengthening classical case finding and treatment of pulmonary tuberculosis patients according to the ongoing DOTS program is worthy to reduce the transmission link of TB in that area.

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DEATHS AMONG TUBERCULOSIS (TB) PATIENTS DISCHARGED FROM A TB HOSPITAL IN GUATEMALA, AUGUST 2010 - JULY 2011

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Tuberculosis (TB) is the second leading infectious cause of death worldwide, with over one million deaths per year. In Guatemala, approximately 6% of diagnosed TB patients die per year. We describe patient outcomes and factors associated with death among patients presenting to one TB hospital in western Guatemala. Using the hospital's electronic database, we retrospectively identified 415 TB patients diagnosed from August 1, 2010 through July 31, 2011 who resided in 5 of the country's 22 departments geographically closest to the hospital. Patient log books were searched in the 5 health area units to which patients were referred upon hospital discharge to determine treatment outcomes. Basic patient demographic data were obtained from the hospital database. Of 415 pulmonary TB patients ranging in age from 10 to 95 years, 247 (60%) completed treatment (of which 181 (73%) were cured), 22 (5%) abandoned treatment, 31 (7%) died either during the hospitalization or during subsequent outpatient treatment, 3 (1%) were transferred to other health areas, 33 (8%) were still in treatment, and 79 (19%) were either not found or had no information on patient outcome. Among the 31 patients who died, the median age was 49 years (range, 21 to 95 years), 10 (32%) were married, 12 (39%) were HIV positive, 17 (55%) worked in agriculture, and 22 (71%) were new cases presenting to healthcare for the first time with symptoms (i.e. not previously diagnosed and already in treatment). Of the 336 patients with a known outcome, 64 (19%) were HIV positive and 12 (19%) died. On multivariate analysis, the odds of dying compared to completing treatment was 5.8 (95% confidence interval 2.4--14.2) times higher for those known to be HIV positive after controlling for age and case type (new TB patient versus previously diagnosed). Continued coordination between HIV and TB programs in Guatemala is needed to diagnose both diseases in a timely manner and decrease mortality.

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FACTORS ASSOCIATED WITH DISAGREEMENT ON RADIOGRAPHIC DIAGNOSIS OF PNEUMONIA BETWEEN WHO AND LOCAL READERS AMONG CHILDREN AGED 2-24 MONTHS IN DHAKA, BANGLADESH

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Diagnosing pediatric pneumonia remains a challenge, even with chest X-rays. The process of reading chest X-rays has been standardized by the World Health Organization (WHO) to improve diagnosis comparability in epidemiological studies, which has shown substantial agreement among readers in validation exercises. However, the consistency of this process in the real world remains unclear. This study aims to compare the level of agreement and factors associated with the disagreement on radiological diagnosis of pneumonia between the WHO panel and Dhaka readers (a radiologist and a pediatrician) who were calibrated to the WHO standardized reading procedure. Consensus and arbitration reading was applied in WHO and Dhaka radiological readings, respectively. Chest X-ray results were analyzed for 2238 children aged 2-24 months who were suspected pneumonia cases recruited in a hospital-based surveillance during August 2000 and April 2003 in Dhaka, Bangladesh. Demographics, antibiotics use and clinical signs were obtained at the time of their hospital visits. Agreement on radiological results interpreted by WHO readers versus Dhaka readers was examined using kappa statistics. Multinomial logistic regression was used to examine factors associated with whether children received both positive, both negative, or discordant readings from the two reader groups. An additional subgroup analysis was performed among children with discordant readings. The agreement between WHO and Dhaka readings was moderate (κ=0.5, 95%CI=0.5-0.6). Factors associated with receiving discordant readings included age, season of hospital visit, antibiotics use, chest indrawing, length of hospital stay and death. Among children with discordant readings only, those who visited hospitals in summer (Odds Ratio (OR)=4.1, 95%CI=2.0-8.3), who were older (OR=1.6, 95%CI=1.2-2.2) and who had dehydration (OR=5.8, 95%CI=1.3-26.8) were more likely to receive Dhaka-positive-only readings. Efforts are needed to modify the diagnostic procedure to improve comparability among different reader groups.

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ETIOLOGY OF SEVERE ACUTE RESPIRATORY ILLNESS IN CAMBODIA, 2010-2011

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Acute respiratory infections contribute to more than 3 million deaths (20% of deaths from all causes) annually worldwide. Many of these infections are potentially preventable with vaccines and treatment. However, etiologies of severe acute respiratory illness (SARI) in middle and low income countries, including in Southeast Asia, are poorly understood. We conducted SARI surveillance in Cambodia to better understand the relative importance of respiratory viruses and determine if TB infection is associated with influenza disease. From January 2010-December 2011 we collected demographic and clinical data from patients hospitalized with SARI (fever, cough, and shortness of breath with onset ≤10 days) at 4 hospitals. We collected nasopharyngeal swabs and sputum samples from these patients and tested specimens for influenza, parainfluenza (PIV), and respiratory syncytial virus (RSV) by reverse transcription-polymerase chain reaction and stained sputum for Acid Fast Bacilli (AFB). Of 1,423 SARI cases, 38% were <5 years old, median age was 24 years, and 43% were female. Influenza was detected in 71 of 1,380 (5%) tested samples and was similarly prevalent in those <5 [20/532 (4%)] and ≥5 years [51/848 (6%)], (p=0.07). RSV was detected in 144/1,162 (12%) SARI cases, 96% were in children <5 years; PIV was detected in 49/1,162 (4%) SARI cases. Of 753 SARI cases tested, 97 (13%) were AFB-positive (presumed TB). Of 717 tested for both influenza and TB, no significant difference was found in frequency of influenza co-infection between those with [4/92 (4%)] and without [41/625 (7%)] TB (p=0.4). Of all SARI cases with known outcome, 30/1,380 (2%) died while hospitalized, of which 15 (50%) were <5 years. Among deaths, 1 was positive for influenza, 2 were positive for PIV, and 5 were positive for RSV; none were AFB positive. RSV was more common than influenza among SARI patients. We found no significant association between influenza and TB among those tested for both. Surveillance can be used to guide prevention and treatment efforts for severe respiratory disease by identifying risk groups and defining common etiologies of illness.

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A COMPARATIVE STUDY OF THE EFFECT HELMINTH INFECTION ON THE INCIDENCE OF ACTIVE TUBERCULOSIS

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Having shown previously that filarial (and other helminth) infections modulate the mycobacterial-specific pro-inflammatory cytokine response necessary for maintaining the latent tuberculous state through the induction of regulatory networks (e.g. IL-10, CTLA-4), we sought to address whether filarial (and/or intestinal helminth) infections alter the progression from latent to active pulmonary tuberculosis (TB) in a cohort of patients followed longitudinally in Tamil Nadu, South India, an area in which *Wuchereria bancrofti* and intestinal helminth infections (primarily hookworm) are co-endemic with TB. A cohort of patients from five villages were assessed at baseline and followed subsequently for six years to study the incidence of development of pulmonary tuberculosis among helminth-infected (*W. bancrofti* and/or hookworm) and non-infected groups. In all, 5096 patients were enrolled from June 1999 to April 2000 at which time, stool examinations, circulating filarial antigen (CFA), tuberculin skin testing were obtained. Patients also underwent chest radiographs and sputum microscopy and culture if they had symptoms consistent with active pulmonary TB. Three subsequent assessments were performed at 2 year intervals; at each visit patients were assessed using tuberculin skin testing and questionnaires related to signs and symptoms of active TB, and -- for those with potential symptoms of TB—sputum microscopy and culture. Of the 5096 patients 1923 were found to be filarial/intestinal helminth infected and 3173 patients were free of helminth infection. 21 patients in each group diagnosed with active TB over the 6 year follow up period for an overall incidence of 1.37/1000 per year. The incidence of pulmonary TB was no different between those who were helminth infected and those who were not (p=0.111 Fisher's exact test). In addition the time to detectability of active TB did not differ between the 2 groups (p=0.666, log rank test using interval censored data). Thus, despite the measurable effect of helminth infection on mycobacterial-specific immune responses, there was little effect of these infections on the clinical progression from latent to active pulmonary TB.

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LARGE MORTALITY DIFFERENCES BETWEEN AUSTRALIAN AND NEW ZEALAND SOLDIERS DURING 1918-19 INFLUENZA PANDEMIC

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The large but variable mortality experienced during the 1918-19 influenza pandemic has not been adequately explained. Military records provide some of the few prospective sources of both morbidity and mortality data from 1918 at the end of the First World War. With a few exceptions, the Australian (Australian Imperial Force, AIF) and New Zealand (New Zealand Expeditionary Force, NZEF) Armies were very similar in training and organization. One of the exceptions was that volunteer Australian recruits were largely trained in England whereas New Zealand had large recruit training camps in New Zealand for conscripts prior to embarkation. The Australian and New Zealand Armies had nearly equal influenza mortality in Europe (6.6 vs. 6.4 deaths / 1000 men) during the 1918-19 influenza pandemic but experienced a nine-fold mortality (1.9 vs. 17.2 deaths / 1000 men) difference in the Southern Hemisphere. Some of the mortality difference can be explained by the earlier arrival of influenza in New Zealand. This striking mortality difference in otherwise very similar military groups is likely to have arisen from their differing training circumstances whereby New Zealand soldiers were newer to the military and thus more immunologically naïve to bacterial respiratory pathogens. These mortality differences in otherwise highly comparable military units highlight the importance of secondary bacterial pneumonia to mortality during influenza pandemics.

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MILD AND ASYMPTOMATIC TRANSMISSION OF INFLUENZA VIRUS A IN PERU

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The World Health Organization recommends passive sentinel surveillance for influenza at health centers and hospitals, but this strategy does not permit detection of mild or asymptomatic infection. We implemented active community-based household surveillance for influenza in 4 ecologically distinct regions of Peru: coastal desert (Lima), dry forest (Tumbes), highlands (Cuzco) and rainforest (Puerto Maldonado). As part of this study, to assess the degree of mild or asymptomatic influenza virus transmission, we conducted a serological survey of 800 persons in randomly selected houses in each of the four cohort sites in July 2011. Serum was tested by the hemagglutination inhibition (HI) test for antibody against influenza virus A/California/07/2009 (pandemic H1N1), A/Perth/16/2009, and A/Brisbane/10/2007. Titers were read as the reciprocal of the highest serum dilution causing complete HI, with ≥1:20 considered positive. All samples were tested in duplicate and results reported as the geometrical mean. Antibody prevalence against A/California/07/2009 was 50.8% (CI:47.4-54.3), 39% (CI:35.6-42.38), 42.1% (CI:38.7-45.6), and 23.8% (CI:20.8-26.7) for Lima, Cusco, Tumbes, and Puerto Maldonado, respectively. Antibody prevalence in Lima was 67.5% (CI:64.2-70.8) and 47.2%(CI:43.9-50.7) against A/Brisbane/10/2007 and A/Perth/16/2009, respectively. Results for the remaining sites are pending. Considering that the attack rate for symptomatic influenza in our Lima cohort population was 10.1%, and that the vaccination rate hovers around 10%, we estimate that 4.2 million people in Lima have been infected with A/California/07/2009 between May 2009 and July 2011 and that 82.5% of the infections were mild or asymptomatic. Very few of these cases would be detected through sentinel surveillance systems. Although, by definition, these infections are associated with low morbidity and mortality, determining the degree of asymptomatic transmission may be essential in understanding the dynamics of influenza virus transmission and designing effective immunization and control measures.

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CHARACTERIZATION OF HUMAN METAPNEUMOVIRUS IN EGYPT

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Human metapneumovirus (HMPV) is a paramyxovirus identified in 2001 causing respiratory infections in children. HMPV was divided into two major genotypes, A and B that are further subdivided into sub-genotypes. Studies indicate that genetic diversity is associated with antigenic variability. Seasonal co-circulation of both genotypes was reported, while some studies suggested the predominance of one genotype per season. Therefore for effective vaccine production, the circulation of HMPV groups should be optimally characterized. Recently, HMPV was detected in Egypt; however, our study is the first to investigate HMPV genotypes in the country. Nasopharyngeal aspirates from 450 patients below 5 years of age with lower respiratory tract infections were collected during 2007. Total nucleic acid was extracted and tested for influenza viruses, parainfluenza viruses, respiratory syncytial virus, adenovirus and HMPV by real time PCR. Partial F and N genes of HMPV were amplified using published primers. Amplified DNA products were purified, and sequenced. Phylogenetic relationships for F and N genes were determined and phylogenetic trees were constructed using MEGA5 software. Of the 450 patients, 40% were below 6 months of age. HMPV was detected in 6.5% of patients, and viral co-infection was found in 31% of positive HMPV samples. HMPV infection peaked in March and was not detected from August through November. Partial F gene segments were amplified from 17 HMPV positive specimens. Analysis of the 450 bp fragment of the F gene identified only genotype B members. Twelve of the 17 samples clustered with the B1 sub-genotype, and 5 samples with B2. Analysis of the 259 bp N gene fragment for 6 samples identified them as members of the B1 sub-genotype. HMPV contributes to lower respiratory tract infections among young children in Egypt. Co-infection with other viruses is common. Population immune pressure mechanisms may explain why only the genotype B was detected among our set of samples; however, our small sample set may also have contributed to this observation.

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RESPIRATORY SYNCYTIAL VIRUS MULTIPLE VIRAL INFECTION AND RISK OF PEDIATRIC PNEUMONIA IN CENTRAL VIETNAM

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Acute respiratory infection (ARI) is the leading cause of mortality and morbidity among children. Respiratory syncytial virus (RSV) is one of the leading respiratory viruses causing pediatric ARI. However comprehensive population-based data on the role of RSV and other respiratory viruses in the development of pneumonia remain largely unclear. We conducted this study to determine the effect of RSV and other respiratory viruses on risk of pneumonia and hospitalized pediatric ARI incidences in Vietnam. Population-based prospective surveillance and case-control study of hospitalized pediatric ARI were conducted in Nha Trang, Vietnam from April 2007 through March 2010. Healthy controls were randomly recruited from the same community. Nasopharyngeal samples were collected and tested for 13 respiratory viruses using multiplex polymerase chain reactions. A total of 1,992 hospitalized ARI episodes including 397 (19.9%) with pneumonia were enrolled. The incidence of hospitalized pneumonia was highest among children under 24 months: 2,171.9 per 100,000(95% confidence interval: 1,947.9 - 2,419.7). The majority of ARI cases (60.9%) were positive for at least one virus. Human rhinovirus (HRV) (24.2%), RSV (20.1%), and influenza A virus (FLUA) (12.0%) were the most common and 9.5% had multiple-viral infections. RSV (RR: 1.3, 1.05-1.59) and human metapneumo virus (HMPV) (RR: 1.72, 1.1 - 2.68) infections independently increased the risk of pneumonia. RSV further increased the risk of pneumonia, when co-infected with HRV, HMPV and Parainfluenza virus-3 (PIV3) (RR: 1.8, 3.9, and 5.7 respectively). The case-control analysis revealed that RSV and FLUA increased the risk of ARI hospitalization (OR: 21.91 and 8.33 respectively) but not HRV. In conclusion, RSV is the leading pathogens associated with risk of pneumonia and ARI hospitalization in central Vietnam.

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GENETIC CHARACTERIZATION OF *CRYPTOSPORIDIUM* AND *GIARDIA* IN CHILDREN IN URBAN SLUM IN NAIROBI, KENYA

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Cryptosporidium spp. and *Giardia spp.* are genera of protozoan parasites that infect a wide range of vertebrates and species within these genera

cause human cryptosporidiosis and giardiasis, which constitute the most common causes of protozoal diarrhoea worldwide, and lead to significant morbidity and mortality in both the developing and developed world. To better understand the transmission of human cryptosporidiosis and giardiasis in Kenya, 1118 faecal samples from children presenting in outpatient clinic I Mukuru slums and 499 from children admitted in Mbagathi District hospital were examined for the presence of *Cryptosporidium* oocysts and *Giardia* cysts using a conventional coproscopic approach. Genomic DNAs from these samples were tested in a nested- PCR-RFLP, targeting regions of the small subunit(SSU) of nuclear ribosomal RNA (for *Cryptosporidium*), and the Glutamatae Dehydrogenase gene (GDH) (for *Giardia*). Subtyping was done by amplifying GP60 and 18S genes for *Cryptosporidium* and *Giardia* respectively followed by direct sequencing. *Cryptosporidium* oocysts were detected in 66 (5.6%) and 73 (14.7%) samples from outpatients and inpatients respectively and *Giardia* cysts detected in 62(5.3%) and 7 (1.4%) samples from inpatients. The RFLP results revealed that *Cryptosporidium hominis* was the most frequently detected species (69(78%) of 88 samples tested), followed by *C. parvum* (13(15%), *C. felis* (4 (4.5%).*C. meleagridis* (1(1%). The results from genotyping of *Giardia* show that there are both the genotype A and B in the study population and the RFLP results show the presence of Assemblages AII (1), BIII (3), BIV (4). Mixed infections were also been observed in 9 samples which have both BIII and BIV as well as one sample that has AII and BIII. These data identify *C. hominis* as the major cause of human cryptosporidiosis in Kenya and suggest anthroponotic as well as zoonotic transmission of the disease. This is the first report of *Giardia* assemblages from patients in Kenya.

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MULTILOCUS GENOTYPING OF HUMAN *GIARDIA DUODENALIS* IN MALAYSIA

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This study was conducted to identify *Giardia duodenalis* assemblages and subtypes prevalent in the aboriginal communities of Peninsular Malaysia. A total of 494 faecal specimens were collected from 494 children living in 13 villages. Of them, 249 were males and 235 were females with the mean age of 7 years. Faecal specimens were examined by microscopy after formol-ether concentration technique and iodine staining. For genotyping, partial sequences of triose phosphate isomerase (tpi), glutamate dehydrogenase (gdh) and b-giardin genes were amplified and subsequently sequenced. Mixed infections of assemblages A and B were detected using tpi-based PCR with assemblages-specific primers. The overall prevalence of G. duodenalis was 17.8% (88/494) based on microscopy. Logistic regression identified drinking piped water as a significant predictor of giardiasis (OR= 2, 95%CI: 1.14 - 3.10). Multilocus genotyping identified assemblages A and B in 38 and 37 samples, respectively. Assemblage-specific protocol based on tpi gene identified assemblages A and B in 1 and 23 samples, respectively, and assemblages A+B in 43 samples. Subtyping based on the three loci showed that all assemblage A isolates belong to sub-assemblage AII. Although, most of assemblage B isolates belong to sub-assemblages BIII and BIV, high genetic polymorphisms were noted making subtyping of some isolates challenging. This study indicates that anthroponotic genotypes/subtypes of *G. duodenalis* are more common in Malaysia, suggesting anthroponotic transmission as the most possible route of transmission for giardiasis.

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COMMON OCCURRENCE OF *GIARDIA DUODENALIS* ASSEMBLAGE A IN ALPACAS

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Giardia duodenalis (syn. *G. lamblia*, *G. intestinalis*) is a common enteric protozoon that infects a wide range of mammal hosts, including humans and domestic animals. A survey is conducted to determine the presence of *G. duodenalis* in alpacas. Fecal samples from 126 alpaca crias up to 30 days of age, and 226 alpacas mother (>2 year old) from three geographic regions in the highland of Peru were analyzed using a nested-PCR was used to amplify a 530-bp fragment of the triosephosphate isomerase (TPI) gene of *Giardia*. All positive samples were genotyped by DNA sequence analysis. Of the 352 fecal samples examined for *Giardia* by PCR, 46 (13%) were found positives. *Giardia* was detected in all geographic regions. The infection rate in alpaca crias and mothers was 33% and 1.8%, respectively. Cohort analysis showed no association of infection between crias and mothers in all areas (p>0.01). There was also no association between *Giardia* infection and occurrence of diarrhea (OR=1.0; p>0.01). Sequence analysis of the TPI PCR products showed the presence of *G. duodenalis* assemblages A and E. The former was seen in 37 animals whereas the latter was seen in nine. Most of the assemblage A infections were caused by the A1 subtype of sub-assemblage AI, except for one, which was caused by the A2 subtype of sub-assemblage AI. All nine infections with assemblage E was detected only in crias from two regions. Assemblage A was found in all three geographic regions, with infection rates of 2.7%, 36.7% and 37.9% in crias respectively. Among the four alpaca mothers positive for *Giardia*, three had assemblage AI and one had assemblage AII. Further studies are needed to address the potential zoonotic transmission of *G. duodenalis* from alpacas.

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THE ASSOCIATION OF THE PREVALENCE OF INTESTINAL PARASITES AND ENVIRONMENT FACTORS IN THE TAPIRAPÉ INDIANS OF THE AMAZON REGION OF MATO GROSSO, BRAZIL

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The prevalence of intestinal pathogens and risk factors associated with their presence were studied in a population of 550 indigenous inhabitants belonging to six Tapirapé villages located in the lower Araguaia Amazon region of Mato Grosso State, Brazil. Four collections were made, two in the dry season, from July 2008 to August 2009 and two during the rainy season, from January 2009 to February 2010. A total of 1526 fecal samples were collected. In the analysis relating the presence of intestinal parasites with the time of the year there was an association between an increase in the incidence of *Ancylostoma* spp., in the dry season ($p < 0.02$) and *Trichuris trichiura* in the rainy season ($p = 0.037$). *E. coli* ($p < 0.001$) and *Sarcocystis* spp. ($p < 0.002$) infections were associated with the dry season while *Blastocystis* spp. ($p < 0.001$), *Chilomastix* spp. ($p = 0.046$), *E. histolytica* / *E. dispar* ($p < 0.001$) and *Giardia intestinalis* ($p < 0.004$), with the rainy season. In the analysis involving individuals who participated in all four collections *Blastocystis* spp., and *E. histolytica* / *E. dispar* were more frequent in the rainy season ($p < 0.001$). In the dry season *Ancylostoma* spp. ($p = 0.003$) and *Chilomastix* spp. ($p < 0.001$) were the most prevalent. The results indicate that environmental factors associated with seasonal variations influence the prevalence of intestinal parasites in humans in this

particular environment. For instance the higher prevalence E. *histolytica* / *E. dispar* and *Blastocystis* spp., during the rainy season suggests they are waterborne and perhaps less resistant to drier conditions. The fact that *Ancylostoma* spp. was most frequent in the dry season is consistent with the reproductive cycle of geohelminths. Its life cycle is more effective in moist soils but the intensity of the rains during the wet season could be having a wash off effect. Besides seasonal variations in the weather other factors also moderate transmission such as the indigenous population's life style in which villagers live in communal homes of more than six individuals. For instance in the wet season they spend more time in their dwellings. This linked to their hygienic habits contributes to increased person-person transmission. Besides this environmental degradation leads to changes in habits which is another factor effecting intestinal parasite transmission in this indigenous community.

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A NOVEL THERAPEUTIC OPTION FOR *BALAMUTHIA MANDRILLARIS* INFECTION

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Balamuthia mandrillaris infection is an uncommon disease characterized by involvement of the skin with subsequent extension to the central nervous system, where it causes granulomatous encephalitis which is almost invariably fatal. No optimal therapy is available for this lethal condition. To report the outcomes of seven patients with *B. mandrilaris infection* treated with a combination regimen of miltefosine, fluconazole and albendazole. A case report is presented. Indirect immunofluorescence staining and PCR using the primer mitochondrial 16SrRNA gene were used to identify *B. mandrillaris* from tissue biopsies. Seven patients are included in this report. Four had granulomatous encephalitis (range of age: 8 to 46 years-old; three of them had in association skin lesions (Two on one of their knees and the other on his nose), and the fourth had rhinosinusitis. The skin lesion was one extensive violaceous plaque, which preceded the neurological involvement (range: 4 - 60 months). The brain MRI features were ring enhancing lesions (one or multiple). A combination regiment including miltefosine (2mg/kg/day), fluconazole (8mg/Kg/day) and albendazole (800mg/day) was initiated after observing compatible histopathology features. Five patients received in addition amphotericin B deoxycholate (total cumulative dose of 25mg/kg); and two patients had a surgical resection of a skin lesion in addition to medical therapy. Four patients had significant improvement and are currently alive with no evidence of active disease after receiving treatment for 6 to 18 months, only one developed neurological involvement. Three patients died after three weeks to 6 months on treatment. Two had extensive centrofacial lesion and granulomatous encephalitis with multiple lesions. Although the prognosis of *B. mandrillaris* infections is still ominous, it seems that is not invariably fatal. The combination regimen of fluconazole, albendazole and the amebicidal drug miltefosine may be included in the limited existing armamentarium for treating free living amoebic infections.

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EVALUATION *IN VITRO* OF THE ANTI *ENTAMOEBA HISTOLYTICA* EFFECT OF TWO FLAVONOIDS: EPICATECHIN AND KAEMPFEROL

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Entamoeba histolytica is the parasite that causes amebiasis, a parasitic infection commonly treated efficiently with metronidazole. However, it has been reported that some ameba strains have become resistant to the drug. Research about new therapies to eliminate *E. histolytica* is an important health priority. We evaluated the *in vitro* anti-amebic activity of two flavonoids, epicatechin and kaempferol, at different times of incubation by spectrophotometric assay. Control samples were incubated with different concentrations of metronidazole and a vehicle. The viability of amebas incubated with epicatechin at 689 µmol/L was diminished by 10, 20 and 30% at 2, 3 and 4 h, respectively. At the same incubation times, the reduction of amebic viability with epicatechin at 1379 µmol/L was 25, 30 and 45%, respectively, and with epicatechin at 2068 µmol/L the reduction was 30, 50 and 53%, respectively. On the other hand, kaempferol at 698 µmol/L diminished amebic viability by 30, 33 and 50% at 2, 3 and 4h, respectively. At the same incubation times, the decrease of amebic viability with kaempferol at 1397 µmol/L was 50, 53 and 55%, respectively, and with kaempferol at 2096 µmol/L the decrease was 60, 70 and 75%, respectively. At similar times, metronidazole at 698 µmol/L reduced the amebic viability by 52, 65 75%, respectively, and at a concentration of 1392 µmol/L the reduction was 70 and 78%. The highest dose of metronidazole (2096 µmol/L) diminished amebic viability by 73, 75 and 78% at 2, 3 and 4 h. In the present work we demonstrated the anti-amebic effect of epicatechin and kaempferol, as well as showing that such an effect is dose- and time-dependent, evidenced by the fact that amebic viability decreased with increasing doses and with a greater time elapsed.

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GIARDIA LAMBLIA GENOTYPING IN CHILDREN AND DOGS FROM A HIGHLY ENDEMIC AMERINDIAN COMMUNITY IN PANAMA

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Giardia lamblia, is the etiologic agent of giardiasis, a gastrointestinal parasitic disease of humans and animals. Giardiasis is an important public health concern among children living in rural and indigenous population in Panama. Genetic characterization of *G. lamblia* isolates has revealed the existence of two groups (assemblages A to B) which are found in humans and in other mammals including domestic dogs. However, the role of these pets in the epidemiology of human infection is still unclear, despite the fact that the zoonotic potential of *Giardia* has been recognized. The present work aimed to evaluate the genetic identity of human and dog *G. lamblia* isolates from fecal samples collected in the indigenous community of Ipetí Choco, Ditrict of Chepo, Panama. After obtaining an informed consent from parents and dog owners, 81 fecal samples from children less than 10 years old and 76 dogs were examined for intestinal parasites by microscopy (formalin-acetate concentration procedure). Of the human and dogs evaluated, 42% (34/81) and 11.8% (9/76) were positive for *Giardia* cysts respectively. DNA was extracted from these positive samples. Genotyping was performed using a PCR-RFLP analysis based on the polymorphisms of the *tpi* and β-giardin genes. Additionally, a real time PCR of the SSU rRNA gene was used. According to the *tpi* and β-giardin genes analysis, the most frequent human genotype was assemblage B (76,6%, 23/30). Assemblage A and mixed infections (AB) were present in one sample (3.3%, 1/30) each one. In dogs assemblage B was found in 2.6% (2/76) and assemblage A in 1.3% (1/76). Using the real time PCR analysis, mixtures of assemblages in individual isolates were commonly observed. Human isolates were identified as AB (46.6%, 14/30); B (36.6%, 11/30) and A (3.3%, 1/30). While dog *Giardia* isolates were characterized as AB (33.3%, 3/9 and B (11.1%, 1/9). Apparently, the frequency of canine giardiasis is low in Ipeti Choco community. However, the zoonotic potential of giardiasis under the observed epidemiological scenario needs further studies.

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ROLE OF REACTIVE OXYGEN SPECIES AND ANTIOXIDANT ENZYME CAPACITY DURING EXPERIMENTAL AMOEBIC LIVER ABSCESS

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In an amoebic liver abscess (ALA), polymorphnuclear cells surround *Entamoeba histolytica* (E. h) amoebas to impede their contact with hepatocytes. Although amoebas in a necrotic area of ALA are usually not viable, parenchymal cells and leukocytes generally suffer damage. This suggests that necrosis could be caused by toxic molecules, including reactive oxygen species (ROS). A decrease in antioxidant enzyme activity may contribute to the extension of parenchymal damage. The present study was undertaken to determine the role of ROS as well as the status of antioxidant enzymes during ALA development. Hamsters were inoculated with E. h. and sacrificed at 12h, 48 h and 7 d of ALA evolution. Control animals were not infected. We determined the percentage of liver lining with lesion, and in liver homogenates measured (by spectrophotometric methods) oxidative stress (TBARS), total antioxidant capacity (TAS), and the enzymatic activity of superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (Cat) in the necrotic area and normal parenchyma. Compared to the control group, the following significant differences were observed in infected animals: a rise in TBARs (P=0.001) and a decrease in TAS (P=0.01104) in the necrotic area at 7 d; an increase (at 12 h) and a decrease (at 48 h) in SOD in the abscess in both the lesion area (P=0.008) and hepatic parenchyma (P=0.009); an elevated (at 12 h; P=0.03) and a diminished (at 7 d; P=0.048) CAT production in the lesion. In necrotic areas, high ROS levels suggest an important role by these molecules in the pathogenesis of ALA. The reduction of TAS and antioxidant activity indicates a failure in defense systems, contributing to the extension of ALA.

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ASSESSING SEROLOGICAL TESTS OF TOXOPLASMOSIS IN PREGNANT WOMEN FROM 2002 TO 2010 IN SENEGAL

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In many African countries including Senegal, toxoplasmosis is not subject of a real understanding. The purpose of this study was to update data on toxoplasmosis antibody prevalence based on antenatal surveillance tests in pregnant women in Dakar, Senegal. The test has been performed in 1310 pregnant women at the laboratory of parasitology and mycology at Le Dantec teaching hospital from 2002 to 2010. immunoenzymatic method in solid phase has been used. To accomplish this evaluation, two serological tests (S1 and S2), using venous blood at 3 weeks of interval, are carried out among these pregnant women. The second serology will allow confirming a toxoplasmosis from an immune response, or a non specific antibody fixation. from the 1255 patients tested, we found a prevalence of 8,7% and 0% for (IgM+IgG-) respectively at serology S1 and S2; 24% and 27,1% for (IgM-IgG+), 13,8% and 11% for (IgM+IgG+). 37% of pregnant women present toxoplasmosis antibody, a progress from previous data collected in Senegal. These data confirm the presence of toxoplasmosis among pregnant women in Dakar.

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INDICATION OF HIGH RISK OF MOTHER-TO-CHILD *TOXOPLASMA GONDII* TRANSMISSION IN GHANA

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Toxoplasmosis is a parasitic disease caused by *Toxoplasma gondii* which can be acquired by ingestion of infective stages of the parasite or congenitally from mother to child. Infection can be acute with tachyzoites in circulation or chronic with formation of cysts in muscles and organs. Acute infection may be primary or as a result of re-activation of chronic infection. Congenital infection of infants is known to result in ophthalmic disorders later in life. Recent research in Ghana revealed high sero-prevalence among pregnant women and eye patients. This study sought to determine the risk of transmission of *T. gondii* infection from mother-to-child among women at delivery in a hospital facility. The study involved 76 pregnant women aged 20 to 45 years who consented to participate. Blood and Tissue samples were taken from the maternal side of each placenta after delivery. Umbilical cord blood samples were also taken after they were separated from the infants. Finger-prick blood was taken from infants from participating mothers two to six weeks post-natal. ELISA was used to detect IgG and IgM antibodies against T. gondii in all blood samples while Nested-PCR was used to detect *T. gondii* DNA extracted from placental tissue. Data collected were analysed using SPSS. Results showed 42.1% (32/76) of maternal blood to be positive for anti- *T. gondii* IgG-ELISA (CTK Biotech, Inc.) with 42% of corresponding umbilical cord blood also being positive for IgG. 32.5% (13/40) of post-natal infant blood was positive for anti- *T. gondii* IgG. All of the same blood samples were negative for IgM. Nested-PCR detected *T. gondii* DNA in 40.2% of placental tissue. 3 (3.94%) maternal blood were positive for IgG but the corresponding placental tissue samples were negative for PCR. The presence of anti *T. gondii* IgG antibodies only and *T. gondii* DNA in placental tissues which could be from cysts indicate the women might have had the infection during the pregnancy. In addition, detection of anti-parasite antibodies in umbilical cord and post-natal infant blood suggests a high risk of congenital transmission of the infection to the infants. These results provide baseline data for future work to ascertain the rate of mother-to-child transmission in Ghana.

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EVALUATION OF INDICATORS OF BOVINE BABESIOSIS IN TICKS *RHIPICEPHALUS (BOOPHILUS) MICROPLUS* AND CATTLE FROM THREE TO NINE MONTHS OF THE COLOMBIAN MIDDLE MAGDALENA

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Babesia sp. is a parasite transmitted by ticks affecting livestock in the world. The Magdalena Medio region of Colombia is enzootic for babesiosis, but no studies that demonstrate the behavior of its transmission cycle. The objective was to evaluate entomological and parasitological indicators of infection with *Babesia* sp. in cattle and ticks in the region, through direct microscopic and molecular techniques. We designed a descriptive study of representative non-probability, the number of calves sampled was 237, to which was extracted from blood and adult female ticks for analysis by direct microscopic and molecular techniques to detect infection *Babesia* sp. We obtained a positive for *B. bigemina* of 59.9% and mixed infection (*B. bovis* + *B. bigemina*) of 3.4% was not found positive for *B. bovis* as a single agent. In ticks, a total of 770 specimens captured, analyzed for hemolymph, the total percentage of ticks positive for *B. bigemina* was 79.2% and 9.4% mixed infection. The total infestation area was 3.2 ticks per calf. The calves of 6-7 months

had the highest degree of infestation with 4.7 ticks per calf. A positive correlation was established between the frequencies of the bath over time ticks and parasite burden in cattle. In conclusion, monitoring is needed of the disease in pastoral areas, with new entomological and parasitological indicators that account for the complexity of fenómeno. These results infer that a higher frequency of acaricide treatments 90 days in stable areas is an abiotic factor that favors the acquisition of protective immunity in calves, a positive influence on the natural control of infection and the absence of disease over time. CODI

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DEGRADATION AND UTILIZATION OF COMPLEX CARBOHYDRATES BY *TRICHOMONAS VAGINALIS*

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Trichomonas vaginalis is a protozoan parasite that is the causative agent of trichomoniasis, a widespread sexually transmitted disease that affects millions worldwide. Several reports suggest that infection with this protozoan correlates with a decrease in the glycogen content of the vaginal epithelium. Most studies of *T. vaginalis* include the maintenance of parasites in media containing either glucose or maltose as carbohydrate sources. Here, we demonstrate that *T. vaginalis* grows equally well in media containing the glucose polymers amylopectin or glycogen as the principal carbon source. Having demonstrated the ability of *Trichomonas* to grow and utilize these polymers to support growth, we sought to analyze cell pellets and culture supernatant for hydrolytic activity towards amylopectin. We hypothesized that *Trichomonas* utilizes glucose polymers by first degrading the polymers into smaller subunits. Our data indicate that *T. vaginalis* possess both cell-associated and secreted hydrolytic activity towards glucose polymers and that activity accumulates in the medium during growth. Furthermore, carbohydrate limitation triggers an increase in both activities. Our initial analysis of the secreted activity reveals enzymatic properties consistent with those of an α-amylase. Collectively, our data provide evidence for a potential role of glucohydrolases in the growth of *T. vaginalis*.

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PENTATRICHOMONAS HOMINIS IS ASSOCIATED WITH DIARRHEAL EPISODES IN CAPTIVE-BRED OWL MONKEYS (*AOTUS NANCYMAAE*)

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Owl monkeys (*Aotus nancymaae*) are small New World, non-human primates found in Brazil, Colombia and Peru. This species has been extensively used in biomedical research in the areas of infectious diseases, glomerulonephritis, atherosclerosis, immunology, and vision research. They have also been established as animal models for diarrhea caused by enterotoxigenic *Escherichia coli*, *Campylobacter jejuni*, and *Shigella flexneri*. Protozoan organisms such as *Giardia intestinalis*, *Cryptosporidium* spp., and *Entamoeba histolytica* are known to cause gastroenteritis in New World primates. On the contrary, Trichomonads and *Blastocystis* spp. are commonly found in these species but normally no clinical signs are observed and treatment is not routinely warranted. However, after a small increase in diarrhea rates at our laboratory facility, we investigated the prevalence of intestinal protozoa in captive-bred owl monkeys and its association with abnormal stool consistency. Four hundred sixty-one stool samples were collected irrespective of stool consistency at the NAMRU-6 Laboratory Animal Facility between 2009 and 2012. Identification of intestinal protozoa was performed by microscopy. Trichomonad species was determined by sequencing the ITS locus. Diarrhea was defined as non-formed stool and association with protozoan presence was analyzed with regression methods. Overall, trichomonads and *Blastocystis* spp. were found in 54% and 39% samples, respectively. Two hundred fifty-eight (56%) samples were classified as diarrhea. The prevalence of diarrhea in animals with and without trichomonads was 72% and 38%, respectively (ratio: 1.91, 95%, $p<0.001$). Similarly, the prevalence of diarrhea in animals with and without *Blastocystis* spp. was 72% and 46%, respectively (ratio: 1.56, 95%, $p<0.001$). In regression analyses, both protozoa had highly significant and independent effects (trichomonad ratio: 1.76, $p<0.001$, *Blastocystis* ratio: 1.34, $p<0.001$). Sequencing analysis of trichomonas organisms found showed 99% homology to *Pentatrichomonas hominis*. While intestinal trichomoniasis is normally non-pathogenic, infection due to *P. hominis* in research non-human primates may result in diarrhea and could influence the outcome of gastroenteritis research studies. Careful evaluation of research animals should be instituted and treatment alternatives should be considered to treat and prevent diarrhea due to trichomoniasis.

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CONGENITAL TOXOPLASMOSIS IN BRAZIL: MODELING THE COST OF MATERNAL SCREENING

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Toxoplasma gondii is a protozoal parasite infecting a high proportion of the world's population, although infection is generally asymptomatic in immunocompetent people. Congenital infection can result in fetal death or mild to profound visual, cognitive, and hearing impairment. A decision-analytic model applying the European protocol of universal maternal screening/ treatment to the low-prevalence US population found cost saving of $1 billion and prevention of avoidable injury in thousands of children every year. Using TreeAge Pro Suite software, we constructed a decision-analytic model to estimate costs of untreated toxoplasmosis and costs of screening, treatment, and follow-up for 3 high-prevalence Brazilian states. The model includes probabilities of maternal and fetal infection, fetal loss due to congenital toxoplasmosis (CT), post-natal infection, distribution of visual, hearing, and central nervous system injury, treatment efficacy, and non-probabilistic variables, such as costs of screening tests and treatment. Brazil has very high prevalence of toxoplasmosis, from 30% to 80% in different states, with different ecologies and quality of water and sanitary infrastructure. High adult prevalence is associated with high incidence during pregnancy due to acquisition in adolescence and young adulthood. High incidence of CT is compounded by a more virulent strain than found in Europe. The Brazilian strain affects 1 in 500 births and also can produce blindness when acquired post-natally, even in immunocompetent persons. Clinical experience in Brazil indicates that the local strain, if untreated, produces more profound injuries than the European strain, but that prenatal treatment is equally effective in preventing or mitigating injury. High levels of exposure, including from the water supply, make pre-natal and post-natal incidence a serious public health problem. In this high-incidence population, maternal screening is found to be cost-saving. Universal screening also has spillover benefits in community education, reducing post-natal infection and visual injury.

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ASSESSMENT OF CRYOPROTECTANT TOXICITY ON THE VIABILITY OF *CRYPTOSPORIDIUM PARVUM* AND EVALUATION OF A CRYOPRESERVATION METHOD FOR SPOROZOITES

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Cryptosporidum parvum is an obligate intracellular parasite that can cause life-threatening infections among immunocompromised individuals. Successful cryopreservation and recovery of viable *C. parvum* has not been achieved in spite of multiple studies. This constitutes an obstacle to the establishment of standardized isolates or cloned stocks. Compounds used as cryoprotectants may have detrimental effects on parasite viability, thus toxicity studies should be pursued before developing any cryopreservation method. The present study assessed the effects of different cryoprotective agents on the viability of *C. parvum* and evaluated the infectivity of sporozoites following cryopreservation. Treatment of *C. parvum* oocysts with ethylene glycol (EG), propylene glycol (PG), dimethyl sulfoxide (DMSO), glycerol, or sucrose did not result in incorporation of propidium iodide at cryoprotectant concentrations ranging from 0.5 to 4 M. In addition, excystation of sporozoites was not impaired by the treatment of oocysts with these compounds. Treatment of excysted sporozoites with EG, PG, DMSO, or sucrose did not affect the infectivity of *C. parvum* as determined by the presence of intracellular meront stages in infected HCT-8 cells. Moreover, the expression of the *C. parvum* genes TRAP, COWP, and 18S rRNA in infected HCT-8 was unaffected by prior treatment of sporozoites with cryoprotectants. Following cryopreservation of sporozoites in a cocktail of DMSO and sucrose, infectivity of HCT-8 cells was observed up to 4 days of infection. In summary, oocysts are highly resistant to potentially toxigenic concentrations of cryoprotectants, an observation that is in all likelihood attributed to the low permeability of the thick cyst wall. In addition, sporozoites are able to withstand concentrations of cryoprotectants commonly used in freezing protocols and are amenable to cryopreservation as assessed by an *in vitro* infection assay. We have established a framework to monitor the concentrations of cryoprotectants and cooling parameters as work continues towards developing an optimal freezing method for the long term storage of *C. parvum*.

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OPPORTUNISTIC INTESTINAL PARASITES AND MALNUTRITION IN MADAGASCAR: HOW TO DESIGN STUDIES?

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Opportunistic intestinal parasites (cryptosporidia, microsporidia, etc.) were mostly described in Europe after emergence of the HIV outbreak but their prevalence fall down dramatically with effective antiretroviral tritherapies. Some of these parasites could affect immune-competent hosts and are described in travellers coming back from their trip with diarrhoea. In tropical countries prevalences of opportunistic parasites seem to be higher than in Europe, but are not well documented. Moreover, in tropical areas other causes of immune-depression can occur like malnutrition or tuberculosis. In Madagascar chronic malnutrition concerns 50% of children fewer than 5 years. Chronic malnutrition could induce immunodepression and opportunists have thus to be intensively researched in these children during diarrhea. To address this link between malnutrition and opportunists, we started studies on four pathogens: cryptosporidias, microsporidias, *Isospora belli* and *Cyclospora cayetanensis*. However the difficulty is to define a technical procedure usable i) to analyse large set of stools collected in the same time on the field, ii) sensitive, specific and at low cost and iii) which do not require trained personal. Quantitative PCR could be the best solution for epidemiological campaigns. However pitfall in definition of PCR is the low number of genes potentially targeting due to the low number of sequences available to do multiple alignment. Moreover in tropical countries water and vegetables can be a huge source of parasites in transit. Overall we choose a three steps procedure for epidemiological studies: Q-PCR / microscopy / sequencing, using ribosomal small subunit sequences. In the same time we setup studies in two hospitals to target under-fed children. A third study was conducted on samples already collected to analyse causes of diarrhea in children leaving in the suburban area of Moramanga (Madagascar). Studies are still in process but associations of cryptosporidias and microsporidias have been already found in several patients.

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ELECTRON MICROSCOPY CHARACTERIZATION OF *DIENTAMOEBA FRAGILIS VIRUS* LIFE CYCLE

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Dientamoeba fragilis is a pathogenic trichomonad parasite found in the gastrointestinal tract of humans and is implicated as a cause of diarrheal disease. The objective of this study was to describe, by transmission electron microscopy, the presence and morphological details of the virus population found in different clinical isolates of *D. fragilis* growing in xenic culture. These virus populations comprise different sizes ranging from 40-200 nm. Their most common shape was spherical, enclosing a dense core, a middle electron-lucent layer and an outer coat. In addition, these VLP populations have an icosahedral capsid structure. The *D. fragilis* VLPs were found in the cytoplasm closely associated with the Golgi complex, with some VLPs budding from the Golgi while other VLPs were detected adjacent to the plasma membrane. These VLPs attach and penetrate into *D. fragilis* by endocytosis and are maintained within vacuoles during batch culture for several daily passages and excreted through exocytosis. Virus-like particles are abundant in the growth media of stationary-phase *D. fragilis* cultures. This is the first study to describe in detail the ultrastructural characteristics of a Dientamoeba fragilis virus (DFV) and its mode of replication in different cultured isolates of *D. fragilis*.

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PERKINSUS MARINUS, THE AGENT RESPONSIBLE FOR DERMO DISEASE IN OYSTERS, DOES NOT INDUCE PATHOLOGY IN HUMANIZED HLA-DR4 MICE AND STIMULATES ORAL IMMUNITY

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Perkinsus marinus (Phylum Perkinsozoa) is a marine protozoan parasite closely related to dinoflagellates (responsible for harmful algal blooms) and apicomplexans (e.g. *Toxoplama*, *Plasmodium*) that has devastated natural and farmed oyster populations in some areas of the USA, significantly affecting the shellfish industry and the estuarine environment. *P. marinus* range extension in the northeastern USA has been associated with global warming, and currently Dermo disease is under surveillance by the World Organization for Animal Health (http://www.oie.int/). The infection prevalence has been estimated in some areas to be as high as 100% often causing death of infected oysters within 1-2 years post-infection. Human consumption of infected oysters is thus likely to occur,

but to our knowledge it has not been investigated in humans or other mammals whether *P. marinus* induces gut pathology or whether oral immunization occurs upon consumption. Here we used a humanized mouse model expressing HLA-DR4 molecules and at the same time lacking expression of mouse MHC-II molecules (C57/BL6 background) to address these questions. Oral feeding with live *P. marinus* PRA240 did not induce pathology as manifested by histological examination of the gastrointestinal tract, lungs, and kidneys. Furthermore, PCR testing showed absence of the oyster parasite in fecal material, indicating that *P. marinus* cannot replicate and/or infect cells in the gastrointestinal tract. Interestingly enough, the humanized mice elicited strong humoral (IgG) and cellular responses to the oyster parasite. Our results thus demonstrate that *P. marinus* does not induce pathology and stimulates gut immunity in HLA-DR4 humanized mice. Ongoing studies are aimed to address whether anti-*P. marinus* immunity can protect humanized mice against malaria.

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A STUDY OF MUSSELS *PERNA PERNA* INFECTED WITH *CRYPTOSPORIDIUM* SPP. INTENDED FOR HUMAN CONSUMPTION INDICATING ENVIRONMENTAL CONTAMINATION

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Sources of contamination such as water drainage of the animal faeces, the use of organic fertilizers and the release of part or untreated sewage contamination favor of various aquatic environments by this parasite since the oocysts are eliminated in the feces of the host. In the seas the presence of *Cryptosporidium* spp. directly affects the quality of fish such as mussels present in the Brazilian coast and is therefore limiting the consumption of food. The study aimed to diagnose and characterize genetically type (s) and/or genotype (s) of *Cryptosporidium* in mussels taken from rocky shores at two locations, Lage Preta and Saco´s Beach, in the Mangaratiba city, State of Rio de Janeiro, performing the sequencing and phylogenetic analyzes, including the deposit of Cryptosporidium sequences from GenBank, to correlate the presence of the parasite with the index of rainfall in the region and to establish possible risks of eating mussels, by identifying the genotype (s) and or species with zoonotic potential. Mussels were collected monthly from March 2009 to February 2010 totaling 12 samples. During data collection, 30 animals were separated from each location and divided into three groups of 10 animals each, totaling 72 samples. For the analyzes, the DNA extracted from tissues of mussels was used in the amplification of sequences *18SSU rRNA* by nested-PCR technique. Results: For species identification, the amplicons were sent for sequencing. During all the study samples was possible to diagnose mussels *Cryptosporidium* positive for at least one of the study sites. It was possible to identify three species *C. andersoni, C. meleagridis* and *C. parvum* in samples obtained from two locations of mussels, by observing the similarity of 99% when compared to existing sequences in GenBank. It is possible the occurrence of human cryptosporidiosis by the consumption of mussels, raw or partially cooked, from the city of Mangaratiba. Statistical analysis showed no influence of rain in positivity of the samples of mussels for *Cryptosporidium*.With these results we conclude that there is likelihood of human exposure through ingestion of mussels from the region studied.

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EPIDEMIOLOGIC ASPECTS OF INFECTION *CRYPTOSPORIDIUM* SPP. IN CALVES DAIRY AND GENETIC CHARACTERIZATION OF SPECIES AND SUBTYPES

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The bovine cryptosporidiosis is caused mainly by four different species, *Cryptosporidium parvum*, *C. bovis*, *C. ryanae* and *C. andersoni*. The first one is of great concern for both livestock and public health. With regard to public health, the species is the subject of several studies due to its high zoonotic potential. The study aimed to: Perform the genotypic characterization of *Cryptosporidium* species and subtypes obtained from fecal samples from calves under one year of age, from dairy farms in the State of Rio de Janeiro, Brazil, establishing the potential for zoonotic species *C. parvum* through diagnosed subtype. Methods: The aim of this study is to determine the occurrence of *Cryptosporidium* species and subtypes in calves up to one year of age, throughout PCR technique usin *18S* and *GP60* as gene target. The occurence of *Cryptosporidium* species in calves up to 1-year-old was determined for 143 animals on three dairy farms on the state of Rio de Janeiro, Brazil. A fecal samples collected directly from each calf rectum was processed to concentrate oocysts using the centrifugal flotation technique in saturated sugar solution before being evaluated microscopically. Results: Of the 28 positive samples in microscopy, 23 were confirmed by Nested-PCR using gene *18SrRNA*. After each PCR-positive specimen was sequenced, the presence of three species of *Cryptosporidium* was observed infecting calves at different ages. Pre-weaned calves were infected with *C. parvum* (7%), whereas post-weaned calves were infected with *C. andersoni* (15%) and *C. ryanae* (1%). All positive samples are being submitted to a second Nested-PCR using gene *GP60* as target. A new sequencing will be made for *C. parvum* positive samples, to observe the most prevalent subtype in the area. Conclusions: Were diagnosed by means of molecular techniques *C. parvum* and zoonotic subtypes, *C. andersoni*, species of importance for dairy production and *C. ryanae*, this species is the first report infecting calves in the state of Rio de Janeiro and the second description of the species in Brazil.

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MALNUTRITION IS ASSOCIATED WITH INCREASED MORTALITY IN ADULT MEDICAL INPATIENTS AT A REGIONAL REFERRAL HOSPITAL IN SOUTHWESTERN UGANDA

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The contribution of malnutrition to the course of acute illness and hospital-based mortality in adults in sub-Saharan Africa (SSA) is not fully described. To determine if malnutrition is associated with increased mortality in hospitalized patients in SSA we conducted a prospective observational study of 318 adult (age ≥ 18 years) medical inpatients admitted to the Mbarara Regional Referral Hospital in southwestern Uganda. For each patient, we calculated body mass index (BMI) and a mini-nutritional assessment short form (MNA-SF) score. We followed patients until death or 30 days from admission. The cohort included 152 (48%) women and the mean (± SD) age was 42 (± 8) years. There were 144 (45%) HIV infected patients and 132 (42%) had suspected tuberculosis (TB). Other diagnoses included severe anemia with HB ≤ 7 g/dl (89, 28%), diarrhea (52, 16%), pneumonia (44, 14%), kidney disease (24, 8%) and stroke (11, 4%). Malnutrition (MNA-SF ≤ 7) occurred in 187 (59%) patients and 149 (47%) patients had a BMI of <18.5 kg/m2. The in-hospital mortality was 18% (57 of 318). Of the 261 patients discharged, only 27 (10%) were lost to 30 day follow-up. The 30 day mortality was 40% (117 of 291). In the univariate analysis, malnutrition, an abnormal temperature (≥38 ºC or <36 ºC), HIV infection, and presence of suspected

TB were significantly associated with mortality ($p < 0.05$). In the multivariate analysis, only malnutrition (adjusted OR 3.7, 95% CI 1.6-8.6, $p = 0.002$) and abnormal temperature (adjusted OR 2.0, 95% CI 1.0-3.9, $p = 0.04$) remained independently associated with mortality. Our findings indicate that malnutrition contributes strongly to mortality in acutely ill hospitalized adults in SSA. Additional research is urgently needed to better understand the pathophysiology of malnutrition in acutely ill hospitalized adults in SSA and ways to mitigate its effect.

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TOXOPLASMA MYOCARDITIS IN IMMUNOCOMPETENT REQUIRING CARDIAC TRANSPLANTATION

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Toxoplasma gondii is a common protozoan parasitic zoonosis with varying prevalence. Toxoplasmosis is predominantly of concern in pregnant women and in immunocompromised hosts either as a primary infection or reactivation. It presents as a self limiting illness in immunocompetent persons but rarely presents as chorioretinitis, encephalitis, polymyositis, pneumonitis, hepatitis and myocarditis. Severe infections are rare because the parassitemia is short lived due to the transformation of tachyzoites into bradyzoites. There were fewer than 50 reported cases of severe toxoplasmosis and 15 cases of myocarditis in immunocompetent subjects worldwide. We report the case of an 18 year old Caucasian immunocompetent female living in tropical Australia who was diagnosed with Toxoplasma myopericarditis causing fulminant heart failure and cardiogenic shock requiring urgent BiVAD implantation as a bridge to cardiac transplantation. Patient did not have a significant travel or exposure history other than to a cat. Prodromal symptoms were very nonspecific but in 4 weeks, patient developed pulmonary oedema and ECG showed widespread ST elevation. Echocardiogram revealed a non dilated left ventricle with an ejection fraction of 20 percent. Toxoplasmosis was diagnosed by EIA levels and the low avidity index of IgG indicating an acute infection. Trimethoprim and Co-Trimoxazole were commenced and an extensive negative test panel excluded other causes. Viruses were not isolated on biopsied ventricular tissue and histopathology did not reveal any micro organisms on special stains. CD3 positive T lymphocyte predominant lymphocytic myocarditis with extensive myocyte necrosis was reported. Patient developed progressive cardiogenic shock requiring urgent BiVAD implantation. Patient underwent cardiac transplantation and device removal 61 days after presenting to hospital. To our knowledge, this is the first reported case of severe myopericarditis secondary to *Toxoplasma* infection in an immunocompetent host requiring a ventricular assist device and cardiac transplantation.

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IDENTIFICATION OF THREE *CANDIDA AFRICANA* STRAINS IN SENEGAL

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Frequency of candidiasis has increased dramatically in recent years. *Candida albicans* is the most common species. However, other species which are pathogenic and resistant to usual antifungal agents beginning to emerge. Among theme *C. dubliniensis* and *C. africana* are the most frequent. These two species presented morphological similarities. Thus, it is important from epidemiological point of view to identify the fungal isolates in Senegal. This study was undertaken to identify new species among *Candida* strains isolated in Dakar. Oropharyngeal and vaginal swabs were performed at Fann Hospital in Dakar. The strains were identified by the germ tube test, the chlamydospore production test and an auxanogram. Then identification by PCR targeting the hyphal wall protein 1(hwp1) gene, was carry out in order to discriminate *C. albicans* between, *C. dubliniensis* and *C. africana*. Among the 243 yeasts, 95% (231/243) were isolated from vaginal swab and 5% (12/243) from oropharyngeal swab. Species identified by phenotypic methods are *C. albicans* which is the most frequent, *C. tropicalis, C. glabrata, C. dubliniensis, C. kefyr and C. lusitaniae*. Of the 150 strains analyzed by PCR, 75% (112/150) were positive. Among the 112 strains of C. sp PCR positive, 97% (109/112) were identified as strains of *C. albicans* and 3% (3/112) as *C. africana*. No strains of *C. dubliniensis* was not found in our study. In conclusion, this study isolates *C. africana* for the first time in Senegal. Further studies on a larger sample will better know the actual proportion of these three species among the isolated yeasts.

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ENTAMOEBA HISTOLYTICA MENINGOENCEPHALITIS IN A SAMOAN TODDLER WITH ACUTE ONSET SEIZURES

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Invasive amebiasis causes extraintestinal disease via hematogenous spread to the liver, lung, brain and rarely other organs. The medical literature reports amebic brain abscesses, which represent advanced infection that is often fatal, but to our knowledge *Entamoeba histolytica* meningoencephalitis has not been described in the modern literature, which is distinct from that caused by free-living amebae. We report what may be the first case of *E. histolytica* meningoencephalitis diagnosed in American Samoa in a 16 month old Samoan boy. Without brain or liver abscesses on CT scan, he developed acute onset seizures while receiving appropriate dose oral metronidazole for intestinal amebiasis and, CSF exam revealed 2+ motile trophozoites. He was treated with intravenous metronidazole for 2 weeks during a complicated ICU course, and had apparent full recovery with no further seizures to date. *E. histolytica* is endemic on the island and is the most common intestinal parasitic infection diagnosed in our lab in the past 2 years. Hence, in American Samoa and other areas endemic for *E. histolytica*, where similar cases might be escaping diagnosis, pediatricians should have a high index of suspicion for CNS amebic infection in a child with intestinal amebiasis and new onset seizures.

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ATYPICAL RABIES: A CASE REPORT FROM A RABIES OUTBREAK IN KENYA

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An outbreak of canine and human rabies occurred in the Kisumu East district of Western Kenya between December 2011 and March 2012, resulting in 369 human exposures, primarily through dog bites. Of these, 201(35.3%) persons began post-exposure vaccination. Two human deaths were reported. Seven (41.2%) of 17 dogs were diagnosed rabid via the direct fluorescent antibody test. One of the cases was a 4½ year-old boy that was admitted at a district hospital on 7th February, 2012 with a 3-day history of verborrhea, hyperactivity, dysphagia, vomiting, fever, and decreased urine flow. The boy acquired a 3-month old puppy 2 months earlier. However, a week before biting the boy, the puppy had been bitten in the head by a stray dog. The puppy died a few days after biting the boy. Despite a negative laboratory test, the boy was treated for cerebral malaria because it is a malaria endemic zone. Later, the diagnosis was changed to meningitis, after the patient developed a stiff neck. On learning of the rabies outbreak in the region, the patient began post-exposure rabies

vaccination, administered on days 0, 3 and 12, but without rabies immune globulin. The patient's condition deteriorated on day 7 with bouts of vomiting. The patient subsequently developed paraparesis and dyspnoea before dying on day 13. Postmortem specimens were obtained from the brain stem, cerebrum and cerebellum. Rabies virus-specific antigens were detected using a direct rapid immune-histochemistry test (dRIT). Although the patient did not have access to intensive care, he survived for two weeks in the hospital whereas typically rabies cases do not survive more than a week without intensive care. This is the first human rabies case to be diagnosed using the dRIT in a developing country. Rabies vaccine administration to the patient after illness was in conflict to existing recommendations and confounded ante-mortem diagnostic testing. The WHO recommendation is to administer the post-exposure vaccine on days 0,3,7,14 and 28. Immune globulins should also be administered.

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A STUDY TO ASSESS INITIAL PARENTAL RESPONSE TO FEVER IN CHILDREN IN MBARARA, UGANDA, A MALARIA ENDEMIC REGION

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Malaria remains one of the leading causes of morbidity and mortality in the developing world, with the greatest burden of which being in sub-Saharan Africa, especially in countries like, Uganda. Traditionally, development of fever in endemic areas has been correlated with malaria, and several cross sectional studies performed in sub-Saharan Africa, have shown that fever is one of the most recognized signs of malaria within the community. It is important to examine parental initial responses to fever as delaying seeking out treatment for malaria and other common causes of childhood fever can be associated with worse outcomes. Our objective was to determine the initial response to fever among parents presenting with febrile children to the pediatric ward at, Mbarara Regional Referral Hospital, in southwestern Uganda. We also aimed to identify factors that determine a parent's initial response to fever. A questionnaire was administered to 74 parents of sick children who presented with a chief complaint of fever. The questionnaire included questions on demographics of the parents and children, and assessed initial parental decisions in response to their child's fever. Despite the fact the majority of the study participants had little formal education and had poor socioeconomic status, the most favored parental response to fever was to utilize a nearby clinic. However only 40% of the respondents reported seeing a healthcare worker within 24 hours of onset of fever. Almost 30% of the respondents reported waiting more than 72 hours to seek out a healthcare worker. The majority of the respondents who did not go to the clinic first reported that they didn't feel the child was sick enough or reported that transportation was a barrier. Given these findings, parents in this region, should be counseled not to delay seeking assistance when their child becomes febrile to help aid in the diagnosis and treatment of malaria, and other potentially life-threatening non-malarial causes of fever such as pneumonia and gastroenteritis.

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ESTIMATION OF THE RIFT VALLEY FEVER BURDEN OF DISEASE IN THE 2006/2007 OUTBREAK IN KENYA

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Rift Valley Fever (RVF) virus causes severe epidemics in livestock and humans resulting in considerable economic losses from disruption of livestock production and market chain and morbidity and mortality in humans. This study estimated the burden of RVF disease in humans using disability adjusted life years (DALYs), assessed human health RVF epidemiological parameters and private and public health costs during the last RVF epidemic in the 2006/2007 in Kenya. We interviewed family members that cared for an infected person in eligible household and key informants in the public health sector in Garissa and Kilifi districts that were heavily affected by the epidemic and at the public health leaders at the national level to assess the private and public health costs. An eligible household was household that had an RVF cases during the 2006/2007 outbreak as identified from the linelist. Secondary data from the Ministry of Health and published literature were reviewed for epidemiological parameters including age and sex categorized incidences, proportions of disease manifestation, and mortality rates in order to compute DALYs using methods developed by the World Health Organization. A total of 127 eligible households were enrolled in to the study with one member interviewed in each household. Those interviewed in these households included 54% males and ranged from 19 to 81 years old with 40 and 45 years as mode and median age, respectively. The RVF virus predominantly infected males during the outbreak with an annual incidence of 0.7 per 1,000 population compared to females at 0.5 per 1,000 population. The burden of RVF during the 2006 and 2007 outbreak was 3.4 DALYs per 1000 population, representing 1% of the total DALYs and estimated household costs of USD120 for every human case reported. In comparison, the total burden of HIV/AIDS and malaria in Kenya is the highest at 24.2% and 7.2% DALYs, respectively. Our results provide vital data on burden of RVF for use by the Government and other institutions to guide in health policy making and resource allocations for prevention and control.

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BURDEN OF PODOCONIOSIS IN EAST AND WEST GOJAM ZONES, NORTHERN ETHIOPIA

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Podoconiosis is geochemical elephantiasis of the lower legs that affects barefoot individuals exposed to red clay soil of volcanic origin. Podoconiosis can be prevented, early forms of the disease can be treated, and disease progression can be curbed. Podoconiosis is an important public health problem in Africa, Central America and northern India. The aim of this study was to assess the burden of podoconiosis in East and West Gojam Zones, northern Ethiopia. A cross-sectional household survey was conducted in two districts covering all 17,553 households in 20 randomly selected *kebeles* (administrative subunits). Following this, detailed structured interview was conducted on all cases identified. The prevalence of podoconiosis in the population aged 15 years and above was found to be 3.3% (95% CI, 3.2% to 3.6%). 87% of cases were in the economically active age group (15-64 years). On average, patients sought treatment five years after the start of the leg swelling. Most subjects had second (42.7%) or third (36.1%) clinical stage disease, 97.9% had mossy lesions, and 53% had open wounds. On average, patients had five episodes of acute ALA per year. The commonest treatment facilities visited were health centers (28.7%) and traditional healers (29.4%). The most common coping measures employed against ALA were staying in bed (55.6%), resorting to less laborious work (44.2%), use of antibiotics (25.8%) and local herbs (20.5%). The median age of first use of shoes and socks were 22 and 23 years, respectively. More men than women owned more than one pair of shoes (61.1% vs. 50.5%; χ^2= 11.6 p=0.001). At the time of interview, 23.6% of the respondents were barefoot, of whom about two-thirds were women. This study showed high prevalence of podoconiosis and associated morbidities such as ALA, mossy lesions and open wounds in northern Ethiopia. Predominance of cases at early clinical stage of podoconiosis indicates the potential for reversing the swelling and calls for disease prevention interventions.

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ACCESS TO TREATMENT FOR CHAGAS DISEASE IN MEXICO: A POLICY ANALYSIS

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The most recent prevalence estimates from the World Health Organization indicate that as many as 1.1 million people in Mexico are infected with *Trypanosoma cruzi,* the etiologic agent of Chagas disease. However, limited information is available about access to treatment for this disease. The aims of this study were to assess the current extent of access in Mexico, analyze the national and state barriers to access, and suggest strategies to overcome them. Morelos was used as a state case study and data were collected from this state and the national Chagas program. Semi-structured in-depth interviews were conducted with 16 key informants and policymakers at both levels. Government policy documents about Chagas disease treatment in Mexico were collected, analyzed to assess treatment access and used to triangulate interview data. Interview responses and information from policy documents were analyzed according to the health systems "control knobs", as defined in the Flagship Framework for Pharmaceutical Policy Reform: regulation, financing, payment, organization, and persuasion. The data showed that 2,847 new cases of Chagas disease were registered nationally from 2007-2011 and in each year but one, the number of new cases was below the national program's target by 11-36%. The Morelos case study revealed that this state made a concerted effort to increase access by purchasing benznidazole, consistent with state responsibility for medicine procurement. The national program mainly coordinated donation of nifurtimox from the WHO Nifurtimox Donation Program. The procurement process used by Morelos was complex and reflected important obstacles at the national level such as exclusion of antitrypanosomal medicines from the national formulary (regulation), exclusion of Chagas disease from the Seguro Popular social insurance package (organization) and limited understanding of the disease by providers (persuasion). The study proposes strategies to overcome these barriers, including adding these medicines to the national formulary and increasing education about the disease.

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RELATIONSHIP BETWEEN NUTRITIONAL STATUS AND THE PREVALENCE OF MALARIA AND ANEMIA AMONG CHILDREN IN THE KASSENA-NANKANA DISTRICT OF GHANA

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Under nutrition, malaria and anemia are significant public health concerns in northern Ghana, especially among children under five years. There is growing interest in the effects of nutritional status on clinical malaria outcomes in pre-school age children. The study objectives were to characterize the effects of indicators of dietary risk on clinical malaria during both the wet and dry seasons. Cases of clinical malaria (wet season n=40, dry season n=42) and matched controls (wet season n=47, dry season n=37) were identified from records of the longitudinal Birth Cohort Study (BCS). Clinical indicators (Hgb, malaria parasitemia, weight) were extracted from study records, and retrospective household questionnaires captured indicators of household hunger, dietary diversity, household food security, and malaria risk behavior. Children from households classified as having very low food security had an increased risk of being clinical malaria cases compared to children from households not classified as having very low food security. (OR=1.78; 95% CI (.92-3.45); p=.087) 17.1% of the matched clinical malaria cases consumed meat compared to only 8.1% of the matched clinical malaria controls. Children who had consumed meat within the 12 month recall period from the administration of the nutritional history questionnaire had an increased risk of being clinical malaria cases compared to children who did not consume meat during the same time period. (OR=3.33; 95% CI (.92-12.11); p=.067) Parasitemia concentrations were significantly higher in cases when compared to controls (Wilcoxon Rank-Sum Test p-value: <.001.); the mean *P. falciparum* density for matched clinical malaria controls was 6,531.3 parasites/ul of blood compared to 42,199.0 parasites/ul of blood for the matched clinical malaria cases. Higher parasitemia levels are statistically associated with clinical malaria case status; very low food security and meat consumption may affect clinical malaria outcomes among children in the Kassena-Nankana District (KND).

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ADDRESSING THE NEGLECTED TROPICAL DISEASE PODOCONIOSIS IN NORTHERN ETHIOPIA: LESSONS LEARNED FROM A NEW COMMUNITY PODOCONIOSIS PROGRAM

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Despite its great public health importance, few control initiatives addressing podoconiosis (non-filarial elephantiasis, a geochemical neglected tropical disease) exist. In June 2010, the first podoconiosis program in Northern Ethiopia, consisting of prevention, awareness, and care and support activities, began in Debre Markos, Northern Ethiopia. This study aims to document and disseminate the lessons learned from a new community podoconiosis program in Debre Markos. We used a content analysis approach to examine and evaluate data from a series of sources. These sources include conducted interview transcripts, a focus group discussion transcript and secondary sources including monitoring and evaluation field reports, observation notes, and research obtained from a literature review. Themes were identified and grouped into matrix tables. Overall, sixteen program steps were identified and grouped into 6 domains: Initial preparation, training and sensitization, foundation building, treatment activity implementation, awareness, and follow-up. Emphasis is placed on the need for baseline data, effective training, local leadership, experience-sharing, mass-awareness, cross-cutting sector issues (i.e. water and waste management), and integration with government health systems. Related successes and challenges are also described, as are stakeholder roles and misconceptions and socio-cultural challenges affecting the program start-up. Many of the identified successes and challenges are relevant to the aim of the podoconiosis program to be sustainable and community-led. Much of this information has already been used to improve the Debre Markos program. We also anticipate that the domains and steps identified will be useful in guiding new programs in other settings where podoconiosis is highly prevalent. We hope to encourage partnerships and collaboration among podoconiosis stakeholders in future growth and disease control expansion.

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CHRONIC LIVER DISEASE IN HEMODIALYSIS

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The functional integrity of the liver is crucial to vitality in normal people and end stage renal disease (ESRD) patients; the prevalence of chronic liver disease (CLD) in hemodialysis (HD) patients differs according to the country or even the region in the same country. Hepatitis C virus (HCV) was recognized as an important cause and consequence of chronic kidney disease. We aim to investigate the prevalence of CLD among HD patients in Tabuk, Saudi Arabia. All HD patients were offered to participate in the

study, interview, medical records including; demographics, family history, and investigation, were the main source of information. Blood samples were taken, before start of HD to detect HBsAg, HCV antibodies and biochemical tests. Abdominal sonar was performed to scan liver, spleen, portal vein and to become aware of ascites. The prevalence of ESRD was 350 per million populations, mean HD duration 40±15 month, mean age 44±18 year, BMI 23±2 kg/m^2, serum albumin 3.2±0.5 (g/dl), males (52 %). Etiology of ESRD was vague in 33% of HD patients, HCV +Ve in 31%, HBsAg +Ve in 6% and 1% +Ve for both, 4% had nonalcoholic steatohepatitis, 3% had compensated liver cirrhosis, 1% ascitis. Most of the CLD patients did not have symptoms. Older age, low education, living in pastoral areas and longer duration of HD appear to be risk factors for CLD in HD patients. Despite no evidence suggests that HD patients are more prone to suffer from hepatic toxic effects than people with normal kidney function, but HD patients usually receive multiple medications; that may have a role in the pathogenesis of drug induced liver disease in this population. Chronic hepatitis C and B among HD patients are mild in disease activity, and are not progressive, perhaps due to immunological abnormalities in HD patients. CLD were more prevalent in HD patients with vague etiology of ESRD so, more extensive investigations are required as HCV, or any CLD may be incriminated in the development of ESRD with vague etiology.

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OUTPATIENT TREATMENT OF SEVERE ACUTE MALNUTRITION AFTER THE PORT-AU-PRINCE EARTHQUAKE: A SINGLE-CENTER REVIEW OF OUTCOMES FROM 2010 AND 2011

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Malnutrition is a major contributor to preventable child mortality in Haiti. Pneumonia and diarrheal disease cause nearly half of all child mortality under age 5 in Haiti, and malnutrition is well-established as a primary risk factor for death from these infections. We conducted a single-center retrospective review of admission and discharge data for children ages 6-60 months who presented to an outpatient treatment program for severe acute malnutrition between April 1, 2010 and December 31, 2011. Recorded data included age in months at entry; neighborhood; gender; grade of edema; mid-upper arm circumference (MUAC); admission weight, height and weight/height z-score; criteria for entry into the program; corresponding anthropometric data at the time of discharge and co-morbid conditions. The records of 1,695 patients were reviewed. Eight patients were excluded due to incomplete discharge data. There were 851 (50%) male patients, 1441 (85%) were between the ages of 6 and 24 months, 234 patients (14%) were referred to a malnutrition clinic closer to their residence and 132 (8%) were admitted for inpatient malnutrition treatment. Of those enrolled, 1083 (82%) children sucessfully completed the outpatient treatment program, while 45 (3%) did not respond to treatment, 31 (2%) abandoned the program, 18 (1%) were transferred to a cholera treatment center, and 131 (10%) were transferred to unspecified medical facilities. Patients identified 21 distinct geographic zones as their current location of residence, the most common being Cite Soleil, Petionville, Croix-des-Bouquets, Delmas, and Tabarre (all within 10km of the program). Among these localities, Croix-des-Bouquets had the highest rate of program completion (84%). Cite Soleil had a lower completion rate than the four other common zones combined (75% vs. 83%, p=0.015). In the two years after the earthquake, this outpatient treatment program achieved recovery rates similar to those previously reported from diverse settings. Root causes for neighborhood differences in rate of program completion are likely multifactorial and include geographic and socioeconomic obstacles to care. These results demonstrate that outpatient treatment for pediatric severe acute malnutrition can be successfully completed in a complex post-disaster setting.

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USING LONGITUDINAL STUDIES TO ESTABLISH NORMAL REFERENCE RANGES FOR HEMATOLOGIC AND IMMUNOLOGICAL PARAMETERS IN HEALTHY PREGNANT WOMAN AND YOUNG CHILDREN IN MALI

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Accurate assessments of hematologic safety profiles and immunological responses to investigational products such as vaccines are often complicated by a lack of normal reference ranges (RR) in the target population, and a poor understanding of how age, gender, genetics, diet and underlying medical conditions influence these parameters. Two specific populations that lack normal RR in Mali are pregnant women and children under five years. We performed a RR study on peripheral whole blood collected from healthy participants enrolled in longitudinal studies, and compared these data to established RR from Uganda and the U.S. We analyzed leukocyte, erythrocyte, and platelet profiles for hematologic RR; and B-cell, T-cell and monocyte subsets for immunological RR. Hematologic profiles varied with pregnancy status, age, gender and geographic location, and the latter suggests a possible genetic effect. Immunological profiles also varied with these parameters, but to a lesser degree. Site-specific normal RR are necessary to accurately establish baseline hematologic and immunological parameters in a target population. In the future, these RR will facilitate the accurate inclusion or exclusion of potential study volunteers, will make the assessment of research-related adverse events more reliable, and will improve the clinical management of patients in Mali.

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INTEGRATING DENGUE AND DIARRHEA CONTROL IN RURAL SCHOOLS IN COLOMBIA: A CLUSTER RANDOMIZED CONTROLLED TRIAL

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Diarrheal diseases and dengue fever are major global health problems. Where provision of clean water is inadequate, water storage is crucial. Fecal contamination of stored water is a common source of diarrheal illness, but stored water also provides breeding sites for dengue vector mosquitoes. Poor household water management is therefore a potential determinant of both diseases. Little is known of the role of stored water for the combined risk of diarrhea and dengue, yet a joint role would be important for developing integrated control efforts. Even less is known of the effect of integrating control of these diseases in school settings. This trial investigates whether interventions against diarrhea and dengue can reduce diarrheal disease and dengue risk factors in rural primary schools

in Colombia. This is the first trial investigating the effect of integrating dengue and diarrhea control interventions and it is also the first trial to do this in school settings. A 2x2 factorial cluster randomized controlled trial is being carried out in rural primary schools in La Mesa and Anapoima municipalities, Cundinamarca, Colombia. Schools were randomized to one of four study arms: diarrhea interventions (DIA), dengue interventions (DEN), combined diarrhea and dengue interventions (DIADEN), and control (C). Schools were allocated publicly in each municipality at the start of the trial. The objective of the trial is to investigate whether these interventions will significantly reduce diarrhea incidence and dengue entomological risk factors. The primary outcome for diarrhea is incidence rate of diarrhea in school children and for dengue entomological risk, *Aedes aegypti* adult density per school. A total of 873 pupils from 34 schools are enrolled in the trial. Here we report results from baseline and the first follow-up data collections.

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A CAMBODIAN DERMATOLOGY NEEDS ASSESSMENT

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There are no epidemiological studies published in the English literature describing the common skin diseases found in Cambodia. Dermatology services are at an early stage of development and, in order to provide information for policy makers and dermatology training course designers a simple needs assessment was undertaken in the environs of Phnom Penh. The aim of the study was to identify the common dermatoses and their impact on those presenting with them. Four different sites were selected; 2 semi-rural and 2 urban. The necessary permission was obtained via Ministry of Health officials. Patients were invited to attend a clinic where they completed an administered questionnaire and were then examined by a team of dermatologists and their diseases recorded. Patients were then given appropriate free treatment. 625 patients were assessed with 76 different diagnoses recorded however the 10 commonest disorders accounted for nearly 60% of the cases with acne, eczema and scabies being the top three. The majority of patients had disease classified as mild to moderate and for a median duration of 12 months. 53% of patients had previously spent an average of $10 on treating their skin disease unsuccessfully. The most likely group of patients to have previously paid to not get rid of their disease was those with scabies (65.3% of those with scabies). The study dermatologists estimated that 97.1% of patients could have been appropriately managed with treatments available in Cambodia. There are no previously published studies assessing the impact of skin disease on patient in Cambodia. Scabies, eczema and acne were the commonest dermatoses with scabies being the most costly to the patient. This supports the notion that educating communities and basic healthcare workers about simple management of common skin diseases with locally available treatment could significantly reduce the impact of dermatoses for the patient and community alike.

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ACCIDENTAL CAUSTIC SODA INGESTION IN GHANA - AN ALARMING AND INCREASING PROBLEM DEMANDING EARLY DETECTION AND INTERVENTION

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The morbidity and mortality associated with accidental chemical ingestion are preventable. A sudden increase in the numbers of children admitted to Okomfo Anokye Teaching Hospital in 2010 following caustic soda ingestion led to a detailed case note review of all admissions with poisoning from January 2009 to June 2010. The purpose of the review was to identify possible causes for the increase and then develop an effective public health strategy to reverse the trend. There was a six fold increase in the number of children suffering from caustic soda poisoning from Jan-Jun 2010 (13) compared with Jan-June 2009 (2), whilst numbers of cases of poisoning for all causes (19 vs 34) poisoning had doubled. The majority of cases were under three years old and males accounted for 50 of the 72 cases. All children received palm oil to induce vomiting. Complications included oesophageal ulceration, aspiration pneumonia and death. A number of hypotheses for these increases are postulated including a new National Health Insurance Scheme (NHIS) leading to an increase in the number of cases who present to health care facilities; a possible increase in the use of caustic soda as a domestic cleaning agent and the introduction of water bottles which are reused to sell and store caustic soda. We favour the last of these hypotheses because the introduction of these drinking bottles has occurred simultaneously to the increase in presentations and we refute the other hypotheses offered. We propose that a public health awareness campaign via radio and text message be used to spread the message of the risk to children of the use of caustic soda and the danger of palm oil as first aid. This should be accompanied by a campaign to restrict and regulate sales of the corrosive chemical.

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INSUFFICIENT IODIZED SALT COVERAGE AT COMMUNITY LEVEL POSES A RISK FOR INDIVIDUAL-LEVEL IODINE DEFICIENCY: THE FOURTH THAI NATIONAL HEALTH EXAMINATION SURVEY 2009

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Thailand was re-classified by the World Bank as an upper-middle income country in 2010. However, iodine deficiency disorder (IDD) remains a significant public health problem due to lack of robust control on salt iodization. This study evaluated the coverage of salt iodization at household and community levels, and their association with iodine deficiency in Thai children. The fourth Thai National Health Examination Survey (NHES IV) was a nationally representative cross-sectional survey conducted in 2009 by three-stage stratified sampling. Children aged 1-14 years were sampled for the study. Children's primary caretaker was interviewed about food intake using a food frequency questionnaire and a 24-hour food recall. Urine iodine of children was tested to measure iodine level. Data were analyzed with descriptive statistics and multi-level logistic regression using R software and epicalc and lmer4 packages. A total of 9035 children were recruited. Nationally, the prevalence of iodine deficiency was 30.8%, with significant regional variations ($p<0.0001$). Multi-level logistic regression showed that individual-level iodine deficiency was significantly associated with insufficient iodine content in household salt (adjusted OR = 1.22; 95% CI = 1.05 - 1.41) and prevalence of insufficiently iodized salt at sub-district level (adjusted OR = 1.44; 95% CI = 1.09 - 1.91). The analysis was adjusted for for weekly consumption of seafood, age, gender, household income, and maternal education. Despite the high success in economic and social development, IDD is still a serious problem in Thailand. The higher strength of association between iodine deficiency and prevalence of insufficiently iodized salt at the community level implies a need for universal coverage of iodized salt, not just iodized salt consumption at the individual household level at present.

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A QUALITATIVE STUDY ON BARRIERS TO CONSISTENT USE OF FOOTWEAR IN WOLAITA ZONE, SOUTH ETHIOPIA: IMPLICATIONS FOR PREVENTION AND CONTROL OF PODOCONIOSIS

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Effectiveness of prevention and control of podoconiosis depends on a community's consistent use of footwear. However, little is known about factors impeding the use of footwear among communities at high risk of podoconiosis through exposure to red clay soil. This study explored the shoe wearing practices of communities in Wolaita, Southern Ethiopia, and identified major barriers to use of footwear with the aim of informing evidence-based preventive strategies. The study was entirely qualitative involving 38 in-depth interviews, 28 focus group discussions and 7 case studies in four selected communities in Wolaita Zone. In total, 307 informants (52 children and 255 adults) participated in the study, using convenience sampling from affected and unaffected segments of the population. Data were coded and analyzed using the NviVO-9 software. Perceiving shoes to have either protective or social value facilitated use of footwear. Although shoe wearing was commonly intermittent, patients were more likely to wear shoes regularly than non-patients. Financial issues, low perceived risk of podoconiosis and lack of access to higher quality protective shoes were major reasons hampering consistent shoe use in the community. Further, lack of sufficient knowledge about the cause of the disease and misconceptions about shoe wearing resulted in irregular use of footwear. Interventions must emphasize changing mindsets about footwear and improving accessibility to protective shoes in the community. Implications of the findings on preventing podoconiosis in Wolaita district are discussed.

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TRAUMA TRAINING COURSES AVAILABLE AROUND THE WORLD: A SYSTEMATIC REVIEW

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Injury deaths are increasing in low-, middle-, and some high- income countries. This burden of disease is greatest in economies that are least equipped to manage trauma care. The availability of trauma training courses to guide management of trauma care throughout the world is not known. We performed a systematic review of English language literature using the search terms "trauma" and "education". In addition, professional colleagues were contacted, and a world-wide web Internet search was completed in an effort to identify all available trauma training courses. 44 courses were identified in total. 71% of all courses identified were developed in high-income countries (HIC); 67% of courses are taught in high-income countries. Of courses implemented in low-middle income countries, 60% of them were developed in HIC. Few courses (14%) are designed exclusively for physicians. Most courses (43%) include health care providers with variable levels of education and training. Trauma care training courses are given throughout the world, many for non-physician providers. It is unknown if additional courses are available yet unidentifiable via our search methodology of scientific publications, internet search, and personal communication. In view of the current global burden of injury, dissemination of training and education in the management of acutely injured patients is essential.

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ANALYSIS OF MEASLES SURVEILLANCE DATA FOR DECISIONS ON SUPPLEMENTARY IMMUNIZATION CAMPAIGN IN SOUTHERN ETHIOPIA

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In 2010, Ethiopian Ministry of Health implemented measles supplementary immunization activity (SIA) campaigns targeting children 9 months-5 years. Despite the campaign, measles outbreaks continued to occur in the Southern regions of the country affecting several districts in the region. Regional epidemiologic and laboratory measles surveillance data from July 2010 to February 2011 i.e. before and after the SIA were reviewed to guide public health decisions to advise the implementation of the campaign. Atotal of 34,782 cases and 64 deaths were reported from 32 of the 125 districts in the region with an incidence of 863 per 100,000 populations and case fatality rate of 0.18%. About 87% (30,366/34,872) of the patients were children below 15 years of age. The incidence was 218 per 100,000 population among children < 5 years and 92 per 100,000 populations among 5-14 years of age before the campaign. After the campaign, the incidence was 346 per 100,000 populations among children age <5years, and 193 per 100,000 populations among 5-14 years of age. The incidence in adults was 12 and 38 cases per 100,000 populations before and after the campaign respectively. The proportion of reported patients from targeted age group was 49% (5,732/11,808) before and 43% (9,110/22,974) after the campaign and proportion from non-targeted age group was 42% (5,017/11,808) before and 45% (10,507/22,974) after the campaign. In conclusion, the 2010 measles SIA decreased the proportion of reported patients from targeted age group after the campaign, but did not prevent further spreading of the outbreak. Changing target age group and schedule follow-up SIAs based on local epidemiology may help to control ongoing outbreaks using such campaigns. Accordingly it is recommended that the SIA started inclusive of age groups beyond

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THE ROLE OF ANGIOGENIC AND INFLAMMATORY FACTORS IN THE PATHOGENESIS OF PREECLAMPSIA

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Pre-eclampsia, a pregnancy complication characterized by hypertension and proteinuria is still a major cause of neonatal and maternal mortality, and acute and long-term morbidities for both mother and neonate. There is mounting evidence that an imbalance between angiogenic factors, such as VEGF (vascular endothelial growth factor) or PlGF (placental growth factor), and inflammatory factors such as interleukin 1 (IL-1) and Tumour Necrosis Factor (TNF) are closely related to the pathogenesis of pre-eclampsia.This study was conducted to determine the role of angiogenic and inflammatory factors in the endothelial dysfunction of the placenta and onward pathogenesis of preeclampsia by measuring and comparing maternal serum levels Ang 1, Ang 2 and Tie receptor 2 with PlGF, sFlt-1 to cytokines IL-1 and TNF. Venous blood would be collected from the Obstetrics and Gynaecology Department of the Korle-Bu Teaching Hospital. The study would involve healthy non-pregnant women, healthy pregnant women and pregnant women between the ages of 16 and 45. The samples would then be centrifuged at room temperature for 15 minutes at 1000 x g. Maternal serum would be analyzed by ELISA for levels of Angiopoietin 1 and 2, PlGF, IL-1 and TNF. It is expected that there would be a correlation between the angiogenic and inflammatory

cytokines in the pathogenesis of preeclampsia which would add to existing knowledge of the syndrome and aid in early diagnosis, treatment and prevention.

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USE OF TELEMEDICINE TO DIAGNOSE RINGWORM IN KENYAN SCHOOL CHILDREN

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Internet-based telemedicine has the potential to alleviate the problem of limited access to healthcare in developing countries. The Mashavu project aims to deploy kiosks that transmit health data and pictures to clinics for analysis by trained personnel. To test this principle, we investigated whether dermatophytic fungal infections (ringworm) could be diagnosed by Kenyan clinicians from pictures of lesions. Six physicians, five physician assistants, and five nurses from Nyeri Provincial Hospital took a test consisting of 15 pictures of KOH prep-confirmed ringworm lesions and 15 pictures of KOH prep negative skin lesions affecting local children. The mean (SD) sensitivity and specificity of ringworm diagnosis for the whole group was 73% (19) and 83% (11) respectively. The physicians had the highest sensitivity and specificity, although only sensitivity reached statistical significance when compared to physician assistants. These results suggest that telemedicine can be used to diagnose simple skin conditions with reasonable sensitivity and specificity.

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HAS TANZANIA EMBRACED THE GREEN LEAF? IMPACT OF AFFORDABLE MEDICINES FACILITY - MALARIA (AMFM) ON ANTIMALARIAL PROVISION IN TANZANIA

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In Tanzania the first line antimalarial is artemisinin based combination therapy (ACT), but uptake remains low. The Affordable Medicines Facility - malaria (AMFm) was launched in 2010 in eight national-scale pilots, to increase access by subsidizing quality-assured ACTs (QAACTs), which have a green leaf logo. We conducted nationally representative surveys of public and private antimalarial outlets before AMFm implementation and one year after to assess impact on QAACT affordability, availability and market share. Here we present detailed results for mainland Tanzania, stratified by rural/urban area and outlet type. This work was commissioned by the Global Fund to Fight AIDS, Tuberculosis and Malaria as part of the AMFm Phase 1 Independent Evaluation. We randomly selected 49 wards at baseline (2010) and follow up (2011), and visited all outlets with potential to stock antimalarials, collecting data on outlet characteristics and stocking patterns from outlets with antimalarials in stock. 3,151and 3,785 outlets were enumerated at baseline and endline respectively, of which 631 and 788 stocked antimalarials and were interviewed. Analysis of results of availability, affordability, and market share. Availability: 78.6% of public health facilities (PHFs) stocked QAACTs compared to 10.7% of private for profit (PFP) outlets. Within PFP outlets, pharmacies were most likely to stock QAACTs (65.3%), compared to less than 10% of drug stores and general stores. Affordability: 78% of QAACTs in PHFs were provided free; the rest had a median price of $0.47 per adult equivalent treatment dose. QAACTs were most costly in PFP outlets (median $4.93), especially in urban areas ($7.04). Among PFP outlets, non-artemisinin drugs, such as SP and amodiaquine, were the cheapest antimalarials. Market share: QAACTs had a market share of 49.1% in urban areas, while non-artemisinin therapy dominated the rural market (78.2%). QAACT market share was very low in PFP outlets (1.0%), compared to 96% for non-artemisinin therapies. These findings will be compared with results at follow up to assess the impact of AMFm on these key indicators.

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HEALTH INFORMATION TECHNOLOGY APPROACHES FOR CONTINUOUS QUALITY IMPROVEMENT OF ANTIRETROVIRAL PROGRAMS IN DEVELOPING COUNTRIES

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Health information technology (HIT) has potential to support continuous quality improvement (CQI) of antiretroviral ARV) programs. The fields of CQI and HIT, however, have yet to become fully integrated. To narrow this gap an assessment of various types of HIT used in CQI was conducted. A comprehensive systematic literature review of PubMed, Google Scholar, ACM Digital Library and IEEE Explore was performed to develop a compendium of HIT approaches for CQI. Resources were included if they addressed the feasibility, implementation, or evaluation of innovative HIT that directly supported CQI of ARV programs or patient outcomes in low and middle income countries. The literature review identified 379 articles addressing HIT and HIV programs or outcomes since 2002; 15% (57) used HIT for CQI. At the programmatic level (n=21), geographic information services, health information management systems and electronic medical records are methods to longitudinally track program and facility characteristics to assess overall performance, determine best practices and facilitate planning. At the clinic level (n=15); electronic medical records and cellular phones provide tools to aide clinical decision-making and more efficiently manage patient information leading to improve patient outcomes. At the patient level (n=21); cellular phones can be used to remind patients about adherence as well as clinic follow-up and electronic adherence monitors can provide a mechanism to remotely monitor and promote ARV adherence. Special consideration must be given to the local context, including the technical expertise and physical infrastructure required to implement, sustain and potentially modify the technology. HIT can facilitate CQI to better inform program planning and support clinical care. When implemented with due planning, these technologies can be powerful tools to longitudinally track the quality of HIV care and facilitate solutions for improvement.

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HEALTH WORKERS IMMUNIZATION STATUS AGAINST HEPATITIS B VIRUS IN BURKINA FASO

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The health workers are among the groups most at risk for infection with hepatitis B. Our study assessed the immunization status against hepatitis B infection among healthcare workers of two health districts of Burkina Faso. We conducted a cross-sectional survey using a self administered questionnaire followed by blood sampling of health workers during August and September 2010. The blood samples were analyzed in the IRSS laboratory to search for anti-HBs antibody. On a total of 462 health workers interviewed, only 59.5% had a immunization card, 47.7% reported receiving at least one dose of HBV vaccine, and only 15.1% had been properly vaccinated (three doses of vaccine according the vaccination schedule). Our results show a variation of the vaccination status according

to sex, age, occupational category and seniority in the profession. The search for anti-HBs antibody (biological markers of immunity against HBV) has shown that vaccinees were significantly better protected (p = 0.01) against HBV infection than those who reported never having been vaccinated (76.7% against 58%). In conclusion, results demonstrate the shortcomings of the infection prevention of occupational health in Burkina Faso. It would be desirable to define strategies that can help strengthening the prevention through routine vaccination of all workers in the health profession.

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VIRTUAL EXPERT PANELS: BRIDGING COMMUNITIES TO PROMOTE EXCHANGE IN GLOBAL HEALTH DELIVERY

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GHDonline.org Expert Panels were designed to foster knowledge exchange among multiple disciplines in an asynchronous online conference. Panels capture practical knowledge and bridge disciplines and professional groups to generate new strategies in delivering health care. Traditionally venues for professional public health exchange have included academic conferences, published literature, and other colloquia. Until recently, few alternatives existed. For global health professionals, the need for a web-based, no-fee forum is paramount. In 2008, the Global Health Delivery Project at Harvard launched GHDonline.org, a virtual platform that now hosts nine public and 51 private communities for over 6,500 health professionals. Members share resources, recommendations, and experiences on diverse topics from how to scale male circumcision for HIV prevention to ventilation design in TB clinics. Each month, GHDonline hosts "Expert Panels," virtual, asynchronous conferences led by experts that users may read and respond to during a two-week window. Given the electronic nature of the conference, GHDonline can track page views, downloads, member contributions, and other statistics. A recent panel, "Strengthening Health Systems - The Role of NGOs," was moderated by leaders including Dr. Agnes Binagwaho, Rwanda's Minister of Health. Over 695 participants from 87 countries representing 472 organizations joined the panel, exchanging 124 commentaries and 23 resources. The data and content from this panel, and all panels, are published in Discussion Briefs, available to all GHDonline users. Expert Panels are an innovative alternative for generating robust discussion, connecting diverse practitioners, and uncovering knowledge from the field. The GHDonline Expert Panel model is transforming the availability and depth of exchange among health professionals, especially those working in remote, resource-limited settings. Insights, perspectives, and experiences shared among experts, at no cost, contribute significantly to the emerging knowledge base in the field of global health delivery.

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KEUR SOCE HEALTH AND DEMOGRAPHIC SURVEILLANCE SITE SYSTEM IN SENEGAL: SITE DESCRIPTION, BASELINE FINDINGS AND POLICY IMPLICATION

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The objective of this study was to analyze baseline results from first phase Demographic and Health Surveillance in Keur Soce Subdistrict, Senegal. To compare results with national and international data and comment on their relevance to health development. Multi-round prospective community based study, Initial Census 2010. Keur Soce is located in rural areas in the region of Kaolack, in the district of Ndiédieng. The area lies between longitudes 16°00'14.8"and 16°07'13"W and latitudes 13°51'53" and 14°00'00"N. It is located at 230 km from Dakar in the Sudano-Sahelian region of Senegal and covers an area of 478 sq. km. The estimated population is 29645 inhabitants and composed mostly of Wolof (90%) and lives mainly on agriculture and livestock. This population is distributed in 73 villages with an average density of 62.7 inhabitants/ km ². Almost all of the area is not electrified, running water (from deep wells) is available in just over half the area, otherwise the water comes from traditional wells.The climate is characterized by the alternation of a long and dry season from November to June and a short rainy season from July to October.The area has a 2 health post and 09 functional health huts. Baseline description of each resident including age, sex, marital status, relationship with HH, education. A full demographic profile was given. The total population is 29,645 inhabitants. Forty two were under 15 years of age. The sex ratio is more pronounced for male than female regarding all age categories, except for the reproductive age group. Over 50% of the population are not married. Thus, the married monogamous represent 20% of the population and married polygamous represent 18% of the population. A small proportion of the population

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PRELIMINARY RESULTS OF A SYSTEMATIC REVIEW OF THE EFFECTIVENESS AND COSTS OF STRATEGIES TO IMPROVE HEALTH WORKER PERFORMANCE IN LOW- AND MIDDLE-INCOME COUNTRIES (LMICS)

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Health workers (HWs) play key roles in improving quality and coverage of health interventions. In LMICs, however, HW performance is often inadequate. Existing reviews of strategies to improve performance are outdated or have important methodological limitations. To characterize the effectiveness and costs of strategies to improve HW performance in LMICs, we conducted a systematic review of 15 electronic databases, 29 document inventories of international organizations, and bibliographies of 510 articles. We focused on studies with methodologically "adequate" designs (eg, trials with comparison groups). After screening, data from relevant reports were double-abstracted and entered into a database. Effect sizes were estimated as absolute changes in performance outcomes. Outcomes included HW practices, patient outcomes, and economic measures. As studies often used different outcomes, we calculated a summary measure: the median effect size (MES) for all primary outcomes from a study. We screened >105,000 citations, and 841 reports met our inclusion criteria. Numerous performance improvement strategies have been studied, usually with multiple components. Most strategies had small MES (<10 percentage-points [%-points]), although some had large effects (>25 %-points). Among eight mutually exclusive strategy groups, MES for most (e.g., training +/- supervision +\- job aids, community activities) were similar (median MES: 7-11 %-points). Job aids alone seemed less effective (median MES = 2 %-points) and strategies that provided commodities seemed more effective (median MES = 17 %-points). Contextual and methodological heterogeneity made comparisons difficult. Preliminary results suggest that the effectiveness of strategies to improve performance varies substantially, with many strategies having small effect sizes. Standardization of methods would facilitate efforts to synthesize the evidence. Additional analyses will identify factors associated with increased effectiveness. Results from this review will inform recommendations on how best to improve HW performance in LMICs.

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THE ECONOMIC IMPACT AND BURDEN OF DENGUE ILLNESS IN NICARAGUA

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Over the past two decades, the number of cases and burden of dengue has steadily risen in Nicaragua, though there have been comparatively few efforts to quantify the economic cost and burden [measured in disability-adjusted life years (DALYs)] of dengue to society. In this study, we utilize source data from the Nicaraguan Ministry of Health (MINSA) to estimate the cost and burden of dengue illness from 1996-2010, including both epidemic and endemic seasons of illness. Costs incorporated both direct costs, which included medical expenditures, prevention campaigns, and vector control costs, and indirect costs, which stemmed from lost productivity secondary to illness. Expansion factors were utilized to account for the large portions of underreported and primarily asymptomatic cases. Monte-Carlo simulations and probabilistic sensitivity analyses were conducted on key parameters in the DALY and costs calculations using primary data from MINSA and other previously published literature values. From 1996-2010, the annual burden of disease ranged from 99-805 DALYs per million, with a mean of 347 DALYs per million, and a majority resulting from classic dengue fever (DF). The total cost of dengue illness ranged from US $5.1-27.6 million per year, with the cost per case ranging from US $125-273, resulting in a per capita cost of US $0.97-5.44 over this study period. This analysis will be important for re-assessment of scarce resources for dengue control in Nicaragua and Latin America, as well as for determining cost-effectiveness of novel vaccine candidates and other therapeutics. Such a comprehensive analytic approach can be easily applied to dengue and similar illnesses in the region to yield a more complete picture of the combined costs of disease to the nation.

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RESEARCH CAPACITY BUILDING IN INDIA: LESSONS LEARNED IN NETWORK COORDINATION, RESEARCH CAPACITY BUILDING AND RESEARCH WITH THE INDOX RESEARCH NETWORK

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INDOX is an academic partnership between Oxford University and eleven of the top cancer centres in India. We work under three domains of activity; network collaboration, research capacity building, and research. Network coordination is administered by a number of permanent staff and through a system of internal governance, annual network meetings, and weekly teleconferences based around 7 cancer site specialty groups made up of specialists from across all the INDOX centres to focus on the specific cancers that are more common in India. Our research capacity building work targets the creation of a network of investigators with sufficient training to conduct multicenter trials. We facilitate research opportunities and award training fellowships to scientists and clinicians from India. Over 100 members of the Network have been awarded fellowships and have attended training courses in Oxford and India. The scheme has covered several areas of clinical research including: early phase trials, protocol design, randomised controlled trials, medical statistics, and good clinical practice. Our research focus is on identifying and answering those questions which address local priorities and in trialling solutions which can be applied locally. As such we are currently conducting a case-control study to investigate the risk factors for common cancers in the India. This study is being conducted across all centres and is the biggest study to date of risk factors associated with cancer in India. Two sections of this study, in breast and colorectal cancers, have already begun and are expected to be complete in two years. The researchers will recruit a 10,000 people newly diagnosed with these two cancers, and a further 10,000 people as healthy controls. As we endeavour to exploit priorities determined from analysis of the epidemiology we have coordinated the centres to participate in investigator initiated and sponsor initiated studies. At each site there is a dedicated INDOX Site Principal Investigator and Site Coordinator, who together form the core of the network. Through the process we have sought to mediate the spend of pharmaceutical companies on trials in India and ensure that trials are aligned with local needs; designed with local ethical knowledge; and executed in such a way that capacity is built in India. We report here on the lessons learnt and the barriers encountered in delivering our tripartite mission in the Indian context.

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STRATEGIES TO MOBILIZE COMMUNITY INCENTIVES FOR THE COMMUNITY DRUG DISTRIBUTORS IN CAMEROON

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In Cameroon, onchocerciasis control uses community-directed treatment with ivermectin (CDTI) that uses community volunteers as drug distributors (CDDs) in the villages. The distribution takes 1-2 weeks per year. One of the challenges is to motivate and retain trained CDDs. In Littoral region, a new strategy was tested to stimulate a greater contribution from the communities to support the CDDs. A 6-step strategy was used: 1) identifying people in charge of organizing the community and clarifying their role and training on the process; 2) helping the community define clear objectives by estimating the cost to support the CDDs; 3) listing the potential sources of support; 4) meeting with identified potential donors (community members, associations, churches, leaders, local NGOs) to discuss the role of the community, giving the estimated budget, and asking for a voluntary contribution; 5) collecting the funds; and 6) reporting to the Health District (HD) and communities the amount collected and their use as managed by the communities. In 2011, more than $4000 were collected in the Littoral region. A survey in 3 HDs with high, medium and low performance on collecting funds showed that the CDD work was recognized as important for the community (96% in 3 HDs). All communities recognized CDD should receive incentives (93, 8%), and most community members would contribute (78%). The difference in the collection of funds was tied to: 1) the way CDDs are selected in communities; 2) communication about the objective of the strategy to mobilize community incentives for CDDs (not only to the decision makers); 3) diversified sources of contributions; and 4) decisions made communally rather than by individual. Most CDDs said that they were motivated to serve their community (34/36), but many mentioned that incentives from the community in money or in-kind support would help (22/36). The lessons learnt and experience gained will be used to improve the strategy for community CDD incentives.

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

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Our study proposes a new strategy, alternative or complementary to the entomological methods based on trapping tsetse flies, to target zones at risk and evaluate tsetse flies control programs in animal african trypanosomosis. It aims to develop a sero-epidemiological tool to assess cattle exposure to tsetse bites. IgG responses against *Glossina* saliva was assessed by ELISA on bovine that were experimentally exposed to tsetse flies and other bloodsucking arthropods in order to detect the cross-reactivities between *Glossina* spp saliva and these arthropods saliva. Only the saliva of Tabanidae spp has cross-reacted with *Glossina* spp saliva. In any case, antibody (Ab) response to *Glossina* spp saliva is transient and decreases within 4 weeks after the stop of experimental exposure. This character is a major advantage to design a biomarker of exposure based on the Ab response to tsetse saliva. Immunoproteomic screening followed by mass spectrometry and bioinformatics tools using has permitted to identify three peptides whose two of Tsal1 (Tsetse salivary gland protein1) and one of Tsal2 (Tsetse salivary gland protein2). These peptides will be produced and validated on bovine serum of CIRDES and PATTEC-Burkina (Pan African Tsetse and Trypanosomiasis Eradication Campaign) in order to develop an easy and reproducible test with higher specificity for the evaluation of *Glossina* exposure.

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LYMPHATIC FILARIASIS MID-TERM IMPACT ASSESSMENT FOLLOWING THREE EFFECTIVE ROUNDS OF MASS DRUG ADMINISTRATION IN SIERRA LEONE

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Lymphatic filariasis (LF) mapping in 2005 using Immunochromatographic Test cards indicated that all 14 health districts (HDs) of Sierra Leone were endemic (prevalence >1%) and needed treatment for LF: 2 had low (1%-4.99%), 1 moderate (5%-9.99%) and 11 high (≥10%) prevalence. Baseline microfilaraemia (mf) studies using the thick blood film method in 2007/8 indicated that prevalence was <1% in 2 districts (1 had 0%), low in 8 and moderate in 2 HDs. MDA with ivermectin and albendazole implemented by community-selected distributors was piloted in 6 HDs in 2007, extended to 12 HDs in 2008 and another 2 HDs in 2010. 4,385,467 (coverage 70.1%), 4,694,711 (coverage 73.2%) and 4,749,556 (coverage 75.1%) people were targeted in 2008, 2009 and 2010 respectively in 12 of the 14 HDs. Geographic coverage of villages/urban areas was 100% in all 12 HDs and program coverage was ≥65.0% in all except for 1 HD that had 59.5% in 2008. A study of mf prevalence was conducted in 2011, 6 months after the last MDA, to determine impact of 3 MDAs on mf prevalence in the 12 HDs. The mf prevalence and density was determined using the thick blood film method. A total of 6,023 people ≥5 years were examined, male 3,170 (52.6%) and female 2,853 (47.4%). Overall mf prevalence was 0.30% (95% CI: 0.19 - 0.47%); population mf density was 0.05 mf/ml (95% CI: 0.03-0.08 mf/ml) and positive-only mf density was 17.59 mf/ml (95% CI: 15.64-19.55 mf/ml). Compared with baseline data, an overall reduction of 87.5% in mf prevalence, 95.5% in population mf density and 65.0% in positive-only mf density ($p<0.0001$) was noted. Mf prevalence reduced to 0.0% (100.0% decrease) in 4 HDs and by 70.0-95.0% in 7 HDs. Only 1 of the 12 HDs still had mf prevalence >1.0% (1.58%) and this district had the highest baseline mf prevalence (6.9%). The results show that after 3 rounds of MDA mf prevalence has decreased to <1.0% in all but 1 of the 12 HDs. The LF elimination programme in Sierra Leone is progressing well and on course to eliminate LF by year 2020 in these 12 HDs.

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PROGRESS TOWARDS CONTROL OF *SCHISTOSOMA MANSONI* INFECTION AFTER THREE ROUNDS OF MASS PRAZIQUANTEL ADMINISTRATION IN SIERRA LEONE

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Five health districts in Sierra Leone were found highly (≥50%) and two moderately endemic for *Schistosoma mansoni*. The remaining 7 districts had no or low endemicity (<10%). MDA with praziquantel (PZQ), mplemented by health workers (HWs), started in 2009 in 6 districts targeting school-going children and treating 562,980 children (coverage 89%). It was scaled up to include all school aged children (SAC) and at risk adults in 7 districts in 2010 treating 1,831,383 persons (coverage 77%) and in 2011 treating 1,781,037 persons (coverage 82%). To minimize side effects expected in heavily parasitized individuals a pre-PZQ meal was funded, implemented by head teachers and/or communities. A survey was performed in 26 sentinel sites in 2012, 9 months after the third round of MDA in the 7 districts. Fresh stool samples from 50 SAC per site were examined by Kato-Katz method for *S.mansoni* infections, recorded as eggs per gram of feces (epg). A total of 1,286 SAC were examined, male 642 (49.9%) and female 644 (50.1%). Overall prevalence was 15.2% (95% CI: 13.3-17.3%) and arithmetic mean intensity of infection was 129epg (95% CI: 105.56-152.97epg). Compared with the baseline data collected in 2008-9, it showed a significant overall reduction of 66.3% in prevalence and 51.7% in intensity of infection ($p<0.0001$). In seven districts, the prevalence ranged from 0.5% (95% CI: 0.0-2.8%) in Bo to 36.0% (95% CI: 26.6-46.2) in Koinadugu. Overall 1.2% of SAC were heavily parasitized (≥400epg) and 3.3% were moderately parasitized (100-399epg), a significant reduction from 8.8% and 18.2% respectively. Twelve sentinel sites were highly endemic in 2008-9 and only 2 sites, Bumbuna in Tonkolili and Sinkunia, in Koinadugu were still highly endemic in 2012. The results suggest that there had been a significant reduction in *S. mansoni* endemicity level across Sierra Leone following 3 rounds of MDA. Effective MDA required planning and coordination at national, district and chiefdom levels, trained and motivated HWs, informed and cooperative communities and their leaders, and monitoring, funding and drug supplies to treat the common side effects experienced by heavily parasitized individuals. Continued targeted MDA is required to achieve the national objective of schistosomiasis control. Maintenance of long term control will require surveillance, education and improved water and sanitation facilities.

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DEVELOPING, MONITORING AND EVALUATING CAPACITY DEVELOPMENT OF CENTRE FOR NEGLECTED TROPICAL DISEASES (CNTD) SUPPORTED LABORATORIES AND STAFF MEMBERS IN ENDEMIC COUNTRIES

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Impact assessment, of interventions against neglected tropical diseases in resource poor settings, requires good technical support through diagnostic laboratories manned by highly skilled technical staff. Many countries in Africa and Asia, endemic for NTDs (Neglected Tropical Diseases), lack the technical capacity and laboratory facilities that will facilitate good practices in impact assessment. To alleviate this problem, Centre for Neglected Tropical Diseases (CNTD) in 2009 embarked on an initiative to strengthen five regional laboratories to support national NTD intervention programmes and to reinforce the capacity of NTD endemic countries staff by training 10 members of staff through PhD studies on epidemiology and integrated control of lymphatic filariasis (LF) in countries of very low capacity, in Africa and Southeast Asia. The main goal of this capacity building effort is to ensure national programmes were able to demonstrate value for money for the funding provided by DFID to eliminate LF as a public health problem. To assist with this mission, CNTD has requested support from LSTM's Capacity Development Impact Research (CDIR) unit to design, monitor and evaluate capacity development of these five laboratories (Ghana, Kenya, Malawi, Sierra Leone and Sri Lanka) and the research activities of the recruited PhD students in line with the activities and outputs specified in the project logframe. Each laboratory and in-country NTD staff will be at different stages of capacity development and they will progress at different rates so each must be monitored separately using both qualitative and quantitative indicators. Capacity development plans will incorporate activities at the levels of individuals, laboratories and national/international context and the plans and indicators will be developed and agreed with all key stakeholders. Although it is anticipated that there will be a need for ongoing inputs to capacity development of laboratories and training of NTD staff members in endemic at all levels, this project will cover the period 1st April 2012 - 31st March 2015.

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IMPROVING NEGLECTED TROPICAL DISEASE (NTD) CONTROL OUTCOMES THROUGH NORTH-SOUTH GLOBAL HEALTH PARTNERSHIPS

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In Tanzania, over 45 million people are at risk of infection with 2 or more of the 5 Preventive Chemotherapy (PCT)-targeted Neglected Tropical Diseases (NTDs) which include Onchocerciasis, Lymphatic Filariasis, Soil Transmitted Helminthiasis, Trachoma and Schistosomiasis. Recognizing the need for increased cost-effectiveness in a resource limited environment, the Ministry of Health and Social Welfare has adopted an integrated approach to NTD control with support from various global organizations and NGOs. In 2009, the program targeted 36 out of 132 districts for integrated Mass Drug Administration (MDA) and in 2010-2011, with support from USAID through RTI/IMA, an additional 44 districts were targeted. In 2012, with increased commitment from multiple partners like USAID/RTI/IMA, DFID, SCI and CNTD, 23 additional districts will be targeted, taking the total coverage to 93 out of 132 districts. With support from USAID, WHO, APOC, RTI and IMA, the number of treatments distributed has increased from 15.4 million in 2009 to 25 million in 2011. These valuable north-south partnerships provide support to local governments and communities to conduct MDA through training, advocacy and supportive supervision. In the 3 years that the integrated approach has been used, MDA trainings have been provided to 115,966 community drug distributors, 23,985 teachers and 11,259 frontline health workers. USAID support has allowed for the allocation of 3 IMA staff with specialized skills to the national NTD secretariat, thereby contributing to capacity building at the national level. To address the shortage of human resources at the national secretariat, IMA staff work jointly with Ministry staff to provide guidance and supervision at all levels of MDA. The various north-south partnerships that have been successfully established are helping to ensure that the NTD program is on the road to national coverage in Tanzania. Establishing similar partnerships in countries where they do not yet exist can further contribute to the control and elimination of NTDs globally.

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DEVELOPMENT AND EVALUATION OF A RAPID DIAGNOSTIC TEST TO SUPPORT ONCHOCERCIASIS CONTROL AND ELIMINATION PROGRAMS

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Onchocerciasis, or river blindness, is a major cause of preventable blindness around the world. Caused by parasitic worms (*Onchocerca volvulus*) transmitted to humans through the bite of the blackfly, onchocerciasis typically affects poor, rural communities near fast-flowing streams and rivers. Mass administration campaigns of the microfilariacide drug ivermectin have significantly reduced the burden of the disease in many mesoendemic and hyperendemic regions to the extent that elimination has become a possibility. Assessment and monitoring of elimination requires rapid diagnostic tests that are sensitive and highly specific for exposure to *O. volvulus*. (Ov). We describe a new rapid diagnostic test (RDT) that detects IgG4 antibodies specific to a previously validated Ov antigen Ov16. To facilitate confirmatory reading of the results and thus quality control of epidemiological survey results, test results must be stable for a long period of time (>24 hours). We describe a novel approach to ensure test result stability on a lateral flow format. Performance data for this test based on panels of well-characterized Ov-infected sera and control sera (including other non-Ov filarial infections) demonstrate that the Ov16-based RDT performs equal to or better than Ov-16-based ELISAs, can be used within whole blood, and has the design properties required (in terms of environmental temperature, humidity exposure profiles, and potential cost) by stakeholders in Ov-endemic regions of Africa and the Americas.

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PITFALLS AND OPPORTUNITIES IN CONTROLLING CO-INFECTIONS

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There is increasing momentum in public health and the veterinary sciences towards a model of integration whereby multiple pathogens are targeted simultaneously. Little is known, however, about the epidemiology of co-infections and strategy for their control is nascent. Using gastro-intestinal nematodes as an example, we construct an epidemiological model that is used to simulate the between-parasite species interactions reported in published field studies and animal models. Many previous studies of nematode co-infections have attempted to infer species interactions based upon infection prevalence data and we demonstrate how this can give rise to spurious results. Uncovering the true nature of between-species interactions is critical in informing control and this is exemplified by the phenomena we describe as 'slaving' and 'release'. 'Slaving' refers to the

tethering of synergistic pathogens co-circulating in a host population. In the situation whereby no effective chemotherapy is available for a particular pathogen, its control can still be achieved by targeting its co-circulating synergist. 'Release' refers to the inadvertent increase in a pathogen's prevalence resulting from the control of a co-circulating antagonist. The success of integrated control programs, therefore, not only rests in the efficacy and spectrum of the available chemotherapeutics, but also in the interactions of the extant pathogen community.

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CONTROL OF NEGLECTED TROPICAL DISEASES IN POST-CONFLICT COUNTRIES IN AFRICA: CHALLENGES FOR LYMPHATIC FILARIASIS ELIMINATION IN LIBERIA

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More than half of the 32 Lymphatic filariasis (LF) endemic countries in Africa are yet to implement mass drug administration (MDA) for the elimination of the disease, 11 years after the Global Programme to Elimination LF (GPELF) was launched in 2000. Majority of the countries that have not started MDA are post-conflict countries like Liberia which has a fragile health system in a resource poor setting recovering from the ravages of war. LF is endemic in 13 out of the 15 counties in Liberia with prevalence of infection ranging from 1-46%. Recent efforts to initiate MDA for the elimination of LF in the country have revealed enormous challenges. Planning for LF elimination on the platform for the integrated control of neglected tropical diseases (NTD) required inter-sectoral collaborations that did not exist. There has been strong resistance to the incorporation of vertical programmes for other NTDs, like onchocerciasis and soil transmitted helminths (STH), into a single integrated national NTD control programme. Liberia also faces logistical challenges for MDA implementation. The 14 year war destroyed technical capacity and physical infrastructure on a massive scale. Nevertheless significant progress has been made to ensure a successful launch of MDA implementation for LF elimination in 2012 using the CDI strategy. A massive social mobilization campaign is planned for hard to reach communities to sensitize them about the benefits of MDA and encourage volunteers to serve as community drug distributors. In this presentation, the baseline data collection, launching and scaling up of MDA activities in Liberia will be described in detail.

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HOW EFFECTIVE IS SCHOOL-BASED DEWORMING FOR THE COMMUNITY WIDE CONTROL OF SOIL TRANSMITTED HELMINTHS?

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The recent London Declaration on neglected tropical diseases was based in part on a new roadmap from WHO to "sustain, expand and extend drug access programmes to ensure the necessary supply of drugs and other interventions to help control by 2020". Drug donations from the pharmaceutical industry form the backbone to this aim, especially for helminth infections. The increased availability of funds to control soil transmitted helminths (STHs) raises the question of how best to use these resources, given that treatment must be administered repeatedly in endemic areas in the absence of improvements in water and sanitation. Deworming for STHs is often targeted at school children because they are at greatest risk of morbidity and because school-based deworming is remarkably cost-effective. However, the impact of school-based deworming on overall transmission in the wider community remains unclear. We examine the effect on transmission by estimating the proportion of parasites targeted by school-based deworming. We use methods derived from the description of the transmission dynamics of the worms, demography and school enrolment, and data from a small number of example settings where age-specific intensity of infection (either worms or eggs) has been measured for all ages. In these settings <30% of the population are 5-15 years old. Combining this demography with the age-intensity profile we estimate that in one setting school children output as little as 10% of hookworm eggs whereas in another setting they harbour up to 50% of ascaris worms (the highest proportion of parasites for our examples). In addition, it is estimated that from 40-70% of these children are enrolled at school. Thus, whilst school-based programmes have many important benefits, the proportion of infective stages targeted by school-based deworming may be limited, particularly where hookworm predominates. We discuss the consequences for transmission for a range of scenarios, including when infective stages deposited by children are more likely to contribute to transmission than those from adults.

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THE BENEFITS OF USING MOBILE PHONES IN MONITORING HEALTH INTERVENTIONS: THE PERSPECTIVE FROM THE NEGLECTED TROPICAL DISEASES CONTROL IN TANZANIA

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Tanzania's health care system is overwhelmed with huge volumes of clients seeking care and served with a handful of qualified staff. This East African nation is ravaged by non-infections and infectious diseases including the Neglected tropical diseases like- lymphatic Filariasis, soil transmitted helminthiasis, Schistosomiasis Onchocerciasis and trachoma, most of which are nonexistent in the developed world. Inefficient service delivery mechanisms resulting from poor record keeping and reporting mechanisms further hamper proper planning and decision-making. The Tanzania Neglected Tropical Diseases (NTD) control program has successfully piloted Mass Drug Distribution (MDA) to over 9,000 at risk population using mobile phone technology synergized with web and desktop applications. Forty (40) community drug distributors (CDDs) were trained and equipped to use mobile phones to conduct house-to-house census, and later distribute Ivermectin and Albendazole to eligible population. The exercise run parallel with the existing/routing paper based census, drug distribution and reporting mechanism. The CDDs were able to quickly acclimatize to QWERTY mobile phone keyboards, learned the mobile application and conducted the census while uploading the data in real time -via internet-- to the central server. With the data in time, the district, regional and national office could calculate drug need and allocate supplied accordingly. Mass drug administration was conducted with coverage report live updates in the central server and via the web. This allowed early intervention decision-making by relevant authorities. Mobile phones provide user-friendly, timely and efficient mechanisms to monitor and evaluate Neglected tropical diseases control activities-e.g. mass drug administration- at the village and sub-village level. In resource-limited setting, they provide a viable solution to data collection and reporting of health interventions programs. The Tanzania experience could be shared in the developing world!

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PATENT EXTENSION: IMPROVING THE FDA'S NEGLECTED TROPICAL DISEASES REVIEW VOUCHER

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A Neglected Tropical Disease (NTD) is a condition that, despite its frequency is not necessarily low, has been for different reasons, especially for affecting the poorest people of the world, submitted to the ostracism of low investment to find better therapeutic options. The burden of disease coming from the NTDs is really high and is very close to other very prevalent conditions in terms of disability-adjusted life years (DALYs). But, none less important is the burden of annual losses on productivity within the low-income countries affected by NTDs. NTDs are not only a health problem, they are also an economic and social problem that is delaying the economies of these countries and why not, the whole world. Therefore, it is important to find the best way to stimulate the funding in the NTDs research arena. Unfortunately, it seems to be that not only good intentions are enough in order to obtain the funds to shorten the pipeline to find new compounds for these diseases. In 2006 a group of academics proposed what today we know as the FDA's NTDs Voucher. The idea is quite simple; if a pharmaceutical company succeeds getting the approval from FDA of a compound for one of the NTDs, this company will obtain a *Priority Review Voucher* (PRV). This means that the time it takes FDA, within the *Fast Track Program* (FTP), to review a new drug application is reduced. The goal for completing a Priority Review is six months. This is supposed to be a tool that can be very useful to put new compounds for NTDs into the market but the outcomes so far are not like they were expected at the beginning. An improved version of the voucher to stimulate the development of drugs for NTDs is proposed. The idea is based on granting a Patent Extension Voucher (PEV) for the companies that achieve in marketing an NTD compound, but taking into account the possibility of second use compounds for NTDs, a demonstrated effectiveness, impact on the targeted NTD and the current advantages of the FDA's PRV. Finally, a way to calculate the value of the proposed PEV is explained.

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DYNAMICS OF HELMINTH INFECTIONS IN EASTERN INDONESIA DURING AND THREE YEARS FOLLOWING A MASS DRUG ADMINISTRATION CAMPAIGN TO ELIMINATE LYMPHATIC FILARIASIS

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The lymphatic filarial parasite *Brugia timori* occurs only in eastern Indonesia, where it causes high morbidity. We evaluated the effect of mass drug administration (MDA) with albendazole and diethycarbamazine in a sentinel, *B. timori* endemic, village on Alor Island in annual surveys over a period of 10 years. Prior to the first round of MDA, the microfilaria (MF) prevalence was 26%, and 80% of the residents had filaria-specific IgG4 antibodies as determined by the Brugia Rapid test. In 2010, 34 months after the 6th and final round of MDA, the MF rate had dropped to 0.2%, and the antibody rate had decreased to 6.4%. The MDA campaign also had a beneficial effect on STH infections. Pre-MDA prevalence rates for *Ascaris*, hookworm and *Trichuris* were 34, 28, and 11%, respectively; these rates were the lowest after the 5th round of MDA with 27, 4, and 2%. Unfortunately, STH rates increased after cessation of MDA and were close to the pre-MDA rates 34 months after the 6th round of MDA. However, intensities of STH infections were still lower than baseline levels, and no heavy infections were detected. This study showed that MDA with DEC/albendazole has had a major impact on B. timori MF and IgG4 antibody rates, and it provides a proof of principle that elimination should be feasible. Our results also documented the value of annual DEC/albendazole as a mass de-worming intervention and emphasize the need for continued STH intervention after cessation of MDA for lymphatic filariasis.

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DISCOVERY OF ANTIGENS FOR DIAGNOSIS OF SOIL-TRANSMITTED HELMINTH INFECTIONS

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The human soil-transmitted helminths whipworm (*Trichuris trichiura*), Ascarids (*Ascaris lumbricoides*), and hookworms (*Necator* and *Ancylostoma*) create a substantial burden for worldwide public health, with an estimated one-third of the world's population infected with one or more of these nematode parasites. The current global strategy to control infections with intestinal nematodes involves mass drug administration of anthelmintic medicines without prior diagnosis. However, cure is often not complete, and the limited variety of available drugs has fueled concerns of parasite resistance. The most widely-used diagnostic method is the microscopic detection of parasite eggs, a labor-intensive technique with inadequate sensitivity and specificity. Therefore a rapid, sensitive, specific, and inexpensive method to detect parasitic worm infections without laboratory infrastructure or trained personnel would offer enormous advantages over current protocols. Using closely-related veterinary parasites, informatic and immunological research efforts have provided strong proof-of-concept that specific and sensitive detection of parasite antigens by ELISA and lateral flow assays is achievable. Controlled timecourse infections in canines show that pre-patent infections are detected and the antigens are quickly diminished following effective anthelmintic treatment. Building upon these data, studies are in progress to clone, express, and purify nematode targets from human parasites. Preliminary results demonstrate that antisera specific to *A. lumbricoides* antigens specifically recognize infected samples with a high level of sensitivity.

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IMPLEMENTING PROGRAMS FOR LYMPHATIC FILARIASIS IN ONCHOCERCIASIS ENDEMIC COMMUNITIES: CHALLENGES TO BASELINE MEASUREMENTS IN ETHIOPIA

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Current (2011) WHO guidelines for launching a lymphatic filariasis (LF) program call first for mapping for the disease using the immunochromatographic card tests (ICT) in areas suspected to be endemic for W. bancrofti. In areas where ICT rates are >1%, sentinel sites are selected for monitoring impact of annual mass drug administration on prevalence of the disease. When microfilaremia in the sentinel sites are <1% (generally by year 5 of MDA), then a Treatment Assessment Survey (TAS) to determine if transmission has been interrupted and MDA can cease. In Ethiopia in 2007, mapping for LF using ICT found the disease to be co-endemic with onchocerciasis in three zones: Bench Maji, North Gondar, and Metekel. MDA with IVM has been ongoing in these Zones since 2004. In 2011, six sentinel sites (two each zone) were selected based on having populations of ≥500 and the highest ICT positivity (range 21%-65%) and baseline testing for Mf was conducted. The total

population in the six sites was 5,337 of which 2,748 were tested for Mf. Results found only 1 mf positive person in Bench Maji (0.11%), 3 in North Gondar (0.17%), and 2 in Metekel (0.12%), all of which are below the 1% threshold expected to be found after 5 years of MDA. Ivermectin is a known microfilaricide and its historic use for onchocerciasis in these zones has likely caused the LF Mf to fall below the 1% threshold. Mf prevalence, however, is a poor indicator for adult W. bancrofti worms and therefore cannot reliably be used to determine whether the adult worm population is still viable. For this reason, and because of the relatively low cost of adding ALB to the existing IVM MDA for onchocerciasis, we have decided to continue with MDA for LF (by adding albendazole to ivermectin) according to the WHO recommended strategy.

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ATORVASTATIN AND ARTEMETHER COMBINATION THERAPY REDUCES INFLAMMATION AND IMPROVES RECOVERY OF MICE WITH LATE-STAGE EXPERIMENTAL CEREBRAL MALARIA

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Plasmodium falciparum infection can cause a diffuse encephalopathy known as cerebral malaria (CM), a major contributor to malaria associated mortality. Despite appropriate anti-malaria treatment using quinine or artemisinin derivatives, CM mortalities may be as high as 30% while 25% of survivors experience neurological complications. Thus, adjunctive therapies are urgently needed to prevent or reduce such mortalities. A number of clinical trials involving potential adjunctive therapies for CM have not proven beneficial and some interventions have been deleterious stressing the need for better understanding of CM pathogenesis and development of effective therapies. Chemokines and cytokines have been implicated in the development of CM and CM associated mortalities. Interferon γ induced protein 10 (CXCL10) was recently found to be associated with fatal human CM in field studies in India and Ghana and linked to severity of other infectious diseases. Mice deficient in CXCL10 gene were partially protected against experimental cerebral malaria (ECM) mortality when infected with *P. berghei* ANKA indicating the importance of CXCL10 in the development of CM. We tested the hypothesis that utilizing synthetic products that reduce or neutralize the excessive production of CXCL10 during CM pathogenesis will increase survival and reduce mortality. Atorvastatin is a widely used synthetic drug that lowers cholesterol levels in blood by blocking the enzyme HMG-CoA reductase and has been shown to specifically reduce plasma CXCL10 levels. We determined the effects of atorvastatin/artemether combination therapy on CM outcome in ECM. We assessed immune determinants of severity of CM, survival, and parasitemia in mice receiving the combination therapy from day 6 to 9 post-infection in infected mice and compared the results with controls. The results showed that treatment with atorvastatin significantly reduced systemic inflammation (lower IL-1α, IL-6, IL-17, CCL4, CCL11, and IL-2), reduced potent anti-angiogenic factor CXCL10 and increased angiogenic factor VEGF production. Treatment of the late-stages of ECM in mice with a combination of atorvastatin and artemether improved survival (100%) over treatment with artemether monotherapy (70%), $p<0.05$. Thus, adjunctively reducing CXCL10 and enhancing VEGF levels by atorvastatin during anti-malarial therapy may represent a novel approach to treating CM patients in the future.

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ANTIMALARIAL PRESCRIPTION PRACTICES IN THREE PUBLIC HOSPITALS LOCATED IN AREAS OF VARYING ENDEMICITY IN UGANDA

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In Uganda antimalarials are often prescribed when malaria is unlikely, a problem that is becoming critical following the adaptation of effective but expensive ACTs as the 1st line treatment for uncomplicated malaria. Less known is the extent of irrational use of anti-malarials among hospitalized patients. We present data on anti-malarial prescription practices among hospitalized children with microscopy results. As part of an inpatient malaria surveillance program data was collected from three public hospitals in Uganda: Tororo (high transmission), Jinja (medium transmission) and Kambuga (low transmission). At each site, a standardized case record form was used to collect individual patient level data, including medicines prescribed during hospitalization. Between Jan to Dec11, 15748 children were hospitalized in all three hospitals; Tororo (4949), Jinja (9202) and Kambuga (1597). Over 97% of patients had a thick blood smear performed. Proportion of hospitalized children with a positive blood smear was 60% in Tororo, 47% in Jinja and 34% in Kambuga. Of children with negative blood smear, 280 (14%) in Tororo, 3003 (63%) in Jinja and 683 (66%) were prescribed an antimalarial. Quinine was the most commonly prescribed anti-malarial among children with positive (94%) and negative (84%) test results. Among children prescribed an anti-malarial, the unadjusted odds of death was higher among those with negativeresults as compare to those with positiveresults (OR1.65 95%CI 1.25-2.17, P <0.001). When underlying diagnosis, severity of illness, age and antibiotic use were adjusted for in logistic regression no significant difference in the odds of death was noted between the two groups. Much as there has been improvement in the proportion of children tested for malaria at the sites, prescription of anti-malarials to patients with negative malaria test results remains unacceptably high at two of the sites. With no clear benefit of this practice there is an urgent need to better understand reasons why clinicians continue to treat patients for malaria even when test results are negative.

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NAMIBIAN MEDICINAL PLANT EXTRACTS AND THEIR MECHANISM OF ACTION AGAINST *PLASMODIUM FALCIPARUM* IN AN *IN VITRO* MODEL

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New medicines for malaria are urgently needed, especially in developing countries where malaria is endemic. Malaria treatment depends strongly on traditional medicine as a source for inexpensive treatment of the disease in these countries. In Namibia, malaria is on the decline and the country is moving towards pre-elimination of the disease. However, some communities preferring traditional medicines and not accepting allopathic medicine may prevent elimination. Ethnomedicines need to be integrated into mainstream malaria case management to achieve malaria elimination by 2020. To do so, they need to be scientifically validated to allow for their safe and effective use. In this study, extracts from indigenous medicinal plants *Vahlia capensis, Nicolasia costata,* and *Dicerocarym*

eriocarpum, were previously shown to contain classes of antimalaria compounds through phytochemical screening. Growth inhibition studies using cellular infection models of *Plasmodium falciparum* 3D7 and D10 were carried out to determine anti-plasmodial effects of the extracts. In addition, mechanistic studies were conducted to determine the mode of action of the three plants. Organic extracts of *V. capensis*, *N. costata* and *D. eriocarpum* showed antiplasmodial activity at concentrations ranging from 50-250 µg/mL. Extracts from *D. eriocarpum* showed the highest activity with an IC_{50} of 63.17µg/mL followed by *V. capensis* and *N. costata* at 93.29µg/mL and 86.63µg/mL, respectively. All the plant extracts inhibited haemazoin accumulation with *D. eriocarpum* exhibiting the highest inhibition. The extracts also inhibited protease activity at the early ring stage where infection of red blood cells was being established and at the trophozoite stage where metabolism of the parasites was increased. These results support the ethno-medicinal uses for these plants as complementary medicine for malaria and provide a basis for further studies to determine the potential of the plants as sources of new antimalarial medicines.

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MALARIA CHEMOPREVENTION IN A HIGH TRANSMISSION SETTING: A RANDOMIZED CONTROLLED TRIAL OF MONTHLY DIHYDROARTEMISININ-PIPERAQUINE VERSUS MONTHLY SULFADOXINE-PYRIMETHAMINE VERSUS DAILY TRIMETHOPRIM-SULFAMETHOXAZOLE VERSUS NO THERAPY FOR THE PREVENTION OF MALARIA

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The burden of malaria remains high for infants in some parts of Africa despite the use of insecticide treated bednets (ITNs). Chemoprevention offers a potential means of reducing the malaria burden in infants, however, optimal drug and dosing strategies are unclear in areas were transmission occurs throughout the year and antifolate resistance is high. A cohort of infants aged 4-5 months were enrolled using convenience sampling in Tororo, Uganda, a rural area with perennial high transmission intensity. Infants received an ITN at enrollment and were followed for all their health care needs 7 d/wk. At 6 months of age, infants were randomized using an open label study design to one of four treatment arms; no therapy, monthly sulfadoxine-pyrimethamine (SP), daily trimethoprim-sulfamethoxazole (TS), or monthly dihydroartemisinin-piperaquine (DP). Study drugs were self-administered at home and continued until the infants reach 24 months of age. The primary end point was the incidence of malaria using passive surveillance between 6-24 months of age or early study termination. Malaria incidence was compared using a negative binomial regression model with measures of association expressed as the protective efficacy (PE=1-incidence rate ratio). Preliminary results are presented here. Of 400 infants enrolled, 393 were randomized to therapy of which; 38 were withdrawn before 24 months of age, 277 are actively being followed and 78 have reached 24 months of age. The incidence of malaria is 5.69 episodes per person year (PPY) among those randomized to no therapy; 5.47 episodes PPY among those randomized to monthly SP (PE=7%, 95% CI -17-26%); 4.32 episodes PPY among those randomized to daily TS (PE=26%, 95% CI 7-42%); and 2.32 episodes PPY among those randomized to monthly DP (PE=60%, 95% CI 48-68%). Preliminary results suggest that monthly SP is not effective at preventing malaria, daily TS is associated with only modest protective efficacy, and monthly DP is the most effective regimen. Final results will be available after Sept. 2012 when all infants reach 24 months of age.

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PRESCRIPTION PRACTICES AMONG PUBLIC AND PRIVATE HEALTH FACILITIES FOR UNCOMPLICATED MALARIA IN RURAL GHANA

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Malaria control continues to rely on the diagnosis and prompt treatment of both suspected and confirmed cases through the health care structures. The irrational use of drugs is a major problem for present day medical practice with consequences as the development of resistance to wide range of medicines, ineffective treatment, adverse effects and an economic burden on the patients and economies. No study has been published in Ghana on the prescription practices of both private and public health providers. This study compared the prescription practices of private and public health care providers in Dangme West District of Ghana from February to July 2011. The study was conducted in a demographic surveillance site located in the Greater Accra region of Ghana, with perenial malaria transmission. It is prospective study of patients presenting at eleven public and five private health facilities diagnosed and treated with an antimalarial. 4247 patients prescribed an antimalarial were recruited, of these, 2606 (61.4%) and 1641 (38.6%) represented public and private provider attendants respectively. 31.5% of the patients from public providers were blood slide or rapid diagnostic test diagnosed and 68.5% clinically diagnosed compared to 33.5% and 66.5% for private provider attendants. The mean number of drugs per prescription was 4.2 for public and 4.0 for private providers. Public care providers were more likely to prescribe more drugs than private providers with a minimum prescription of 1 for both providers and a maximum of 10 and 7 respectively. Both providers prescribed an analgesic, antibiotic and a multivitamin as top three drugs in addition to an antimalarial. In conclusion, although malaria treatments were varied, there were not great differences between the public and private providers. Monotherapy with artemisinin derivatives were relatively common. A mishmash of regulatory and non-regulatory interventions, aimed at providers and consumers must be implemented to improve diagnosis and prescription practices of health care providers.

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CLINICAL EFFICACY AND SAFETY OF ARTESUNATE-AMODIAQUINE AND ARTEMETHER LUMEFANTRINE FOR THE TREATMENT OF UNCOMPLICATED MALARIA AND PREVALENCE OF DRUG RESISTANCE MARKERS IN NGAOUNDERE, NORTH CAMEROON

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Cameroon switched policy for treatment of uncomplicated malaria to antimalarial to artesunate-amodiaquine (AS-AQ) and fixed dose artemether-lumefantrine (AL) respectively in 2004 against a backdrop of amodiaquine treatment failure. After four years of implementation of this drug policy, country-specific evidence-based data to support the continuous efficacy and safety profile of ACTs are still needed. This study was carried out in collaboration with the national malaria control program to generate data to support the continuous use of ACTs for malaria case management. A randomised open label trial was conducted between September and December 2007 at the Ngaoundere Protestant Hospital,

Ngaoundere, Cameroon. One hundred and fifty patients between six months to 14 years of age with uncomplicated malaria were randomized to receive standard doses either AS-AQ (73) or AL (77) and followed up for 28 days according to WHO 2003 protocol. Drug safety was evaluated using standard clinical and laboratory parameters and safety concerns classified according to the common toxicity criteria. Response was classified according to WHO and isolates were genotyped for the *msp-2* gene to determine recrudescent parasites. Pre-treatment blood samples were used to determine the prevalence of resistant mutations in the *pfcrt*, *pfmdr1*, dhfr and *dhps* genes by sequencing. Ethical and administrative clearances were obtained from the National Ethics Committee and the Ministry of Public Health in Cameroon respectively. PCR-corrected cure rates were 100% for AL, and 96.4% for AS-AQ. The combinations were well tolerated clinically and biologically. By Day 14, the mean total bilirubin, creatinine and ALAT values were slightly increased in subjects treated with AS-AQ. Changes in white cell counts and platelet count were significantly different ($p < 0.05$) in the two drug groups, but were of no clinical significance. All side-effects were transient and therefore disappeared by the end of treatment. Both AS-AQ and AL are highly effective and well-tolerated for the treatment of uncomplicated *falciparum* malaria in Ngaoundere, Cameroon supporting their continuous use. High prevalence of mutant *pfcrt and pfmdr1* alleles confirm long standing North to South increase in high level CQ resistance and might compromise AQ use in combination therapy. Long-term monitoring of safety and efficacy and molecular markers is however, highly solicited.

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PATTERN OF HEALTH SEEKING BEHAVIOR FOR PREVENTION AND TREATMENT OF MALARIA IN BANDA SLUM, KAMPALA, UGANDA

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Children in slum communities are vulnerable to malaria compared to ones in rural areas. With fever slum residents seek health care in private facilities, health institutions or do not seek care. There was no such data about slums in Uganda. This study assessed the health seeking behavior for respondents with children aged 6-36months in Banda slum Kampala. In February 2009, by cross sectional study interviewed 449 respondents who ≥ one child aged 6-36 months in Banda parish. We asked about where they seek health services, accessibility, reasons for choice, satisfaction, malaria treatment given in case of fever, and where the child aged 6-36 months was treated when s/he had fever 2 weeks prior to the interview. Ethical approval was provided by the Makerere University School of Public health and Uganda National Council of science and technology. Findings are presented as frequencies and percentages. Population characteristics: Of the 449, 416(92.7%) household respondents had only one child aged 6-36 months. Education of the 316 respondents, was 190(60.13%)≤ P.7, 105(33.23%) S1-S4 and 21(6.65%) >S.4. Utility of Health Services:Of the 449, 315(70.5%) sought treatment from a private clinic or a drug shop, 122(27.17%) from a health centre or hospital. Determinants for the choice of health facilities: Of the 396, 233(58.84%) near home, 79(19.95%) had skilled staff, 19(4.8%) drugs available and 65 (16.41%) treatment affordable. Level of satisfaction with the health services: Of the 432, 337(78%) were satisfied, 95(22%) were dissatisfied. Reason being dissatisfied in 86 was inadequate medicines 36(41.9%), expensive 36(41.8%), unavailability of staff 9(10.5%) and long queues 5(5.8%). Mosquito net use:Of 449, 304(67.71%) had mosquito net, 282/(62.9%) children slept under a mosquito net a night prior to the interview. Treatment for malaria of 229 was with chloroquine 53(23%), quinine 65(28.4%), Coartem 20(8.73%), and 6 (2.62%) herbal medicine. Conclusions: Provide training, ACT drugs and diagnostic tests through a public-private partnership in return for subsidized patient charges. Investigate for typhoid as a differential.

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SAFETY OF ARTEMISININS DURING EARLY PREGNANCY, ASSESSED IN 62 SUDANESE WOMEN

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Between June 2006 and October 2008, the safety of artemisinins during early human pregnancy was assessed in central-eastern Sudan. Pregnant women in the first or second trimester who were attending antenatal-care clinics at the Wad Medani, Gadarif and New Halfa hospitals were interviewed. Each was asked if they had had malaria in the first trimester of the index pregnancy and, if so, what treatment they had received. The women who had received artemisinins were then followed-up until delivery and their babies were followed-up until they were 1-year-olds. Overall, 62 of the pregnant women reported receiving artemisinins - artemether injections (48), artesunate plus sulfadoxine-pyrimethamine (11) or artemether plus lumefantrine (three) - during the first trimester. Medical records were available for 51 (82%) of these 62 women, and, in each case, these records showed the reported treatment and that malaria had been confirmed. Only nine (15%) of the 62 women given artemisinins had not known that they were pregnant when treated. Two of the treated women (both given artemether injections in the first trimester) had miscarriages, one at 20 weeks of gestation and the other at 22 weeks, each while receiving quinine infusions for a second attack of malaria. The other 60 women who had received artemisinins delivered apparently healthy babies at full term. No congenital malformations were detected, there was no preterm labor, no maternal deaths were recorded during the follow-up, and none of the babies died during their first year of life. It therefore appears that artemisinins may be safe to use during early pregnancy, although further study is clearly needed.

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A MARKOV MODEL TO EVALUATE THE COST-EFFECTIVENESS OF DIHYDROARTEMISININ-PIPERAQUINE VS. ARTEMETHER-LUMEFANTRINE FOR FIRST-LINE TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN AFRICAN CHILDREN

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Recent randomized multi-center trials showed that dihydroartemisinin-piperaquine (DHAPQ) is as efficacious as artemether-lumefantrine (AL) in treating uncomplicated malaria in African children in different endemicity settings, with comparable safety profiles. The study results also indicate that DHAPQ has a longer post-treatment prophylaxis effect than AL, thus reducing the risk of re-infection following treatment and averting morbidity and mortality. The objective of our economic evaluation is to compare the health outcomes and costs of treatment with DHAPQ or AL as first line therapy in children below six years of age with uncomplicated malaria, in view of the differing post-treatment prophylactic effect profiles of these two drugs. We developed a Markov model to simulate the effectiveness of the two treatment strategies in a hypothetical cohort of 1,000 children over a one-year period. Monte Carlo simulation is used to account for uncertainty in model parameters. The preliminary results of our model show that the estimated number of cases of acute malaria illness are 1545.9 (95%CI: 1543.3--1548.5) and 1716.4 (95%CI: 1713.4--1719.4) per 1,000 children over one year when treated with DHAPQ or AL as first line therapy, respectively. The estimated number of severe malaria infections per 1,000 children are 25.3 (95%CI: 24.8--25.9) with DHAPQ and 28.1 (95%CI 27.5--28.7) with AL treatment in a scenario

where 90% of children with recurrent infections have access to early treatment for uncomplicated malaria. The number of deaths are estimated to decline from 17.6 (95%CI: 17.2--18.1) to 16.0 (95%CI: 15.6--16.4) per 1,000 children over a one-year period when AL is substituted with DHAPQ as first-line therapy. We conclude that even though the post-treatment prophylactic advantage of DHAPQ seems to be relatively small, changing the first-line therapy of uncomplicated *falciparum* malaria from AL to DHAPQ has the potential to significantly reduce malaria-associated morbidity and mortality and thus may provide substantial benefit to the population and appears to be cost-effective when the costs of the drugs are the same.

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ASSESSING THE EFFECT OF THE RECOMMENDED ARTEMETHER-LUMEFANTRINE DOSING REGIMEN ON THE RISK OF TREATMENT FAILURE IN PATIENTS DIAGNOSED WITH UNCOMPLICATED *FALCIPARUM* MALARIA

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Artemether-Lumefantrine (AL), the first line antimalarial treatment in 49 countries, is administered according to four weight bandings, patients at the margins of which deviate significantly from the optimal target dose. To assess the efficacy of administered lumefantrine through the total mg/kg spectrum, individual patient level data (N=8,927) from 43 clinical efficacy studies of uncomplicated *Plasmodium falciparum* treated with 6 doses of AL conducted between 1996 and 2011 (7,399 from Africa; 1,588 from Asia) were collated using standardised procedures. Factors associated with PCR adjusted efficacy were evaluated using Cox regression model with shared frailty to account for study effects. 24 studies ended follow-up at 28 days while 19 studies followed up for 42 days or longer. 192 recrudescent and 1,101 new infections were reported. The median total dose of lumefantrine administered was 65.5mg/kg [IQR: 55.4-77.8 mg/kg], with children under 1 year receiving the greatest dose (median=90.0mg/kg, IQR=80.0-102.9 mg/kg), compared to those aged 1-5 (median=65.5mg/kg, IQR: 55.4-80.0 mg/kg), 5-12 (median=72mg/kg, IQR: 65.5-84.7 mg/kg) and greater than 12 years (median=54.3 mg/kg, IQR: 48.0-62.6 mg/kg). The median mg/kg dose of lumefantrine in patients failing the treatment was 65.5 mg/kg (IQR: 57.5-79.3 mg/kg) which was similar to those who were cured (median=65.5, IQR: 55.4-77.9 mg/kg). In the multivariate model, the risk factors for recrudescent failures were: low weight category (AHR=2.1 [1.1-4.1], *P=0.0330*) and logged baseline parasitaemia (AHR= 1.1 [1.0-1.2], *P=0.048*). After controlling for confounding factors the dose of lumefantrine administered was not associated significantly with treatment failure. Current dosing strategies of AL are robust, but will need careful monitoring particularly as drug resistance to either partner drug emerges and spreads. The WWARN data repository provides an excellent format for this timely and global monitoring

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TREATMENT EFFICACY OF ARTESUNATE-AMODIAQUINE TREATMENT REGIMENS FOR UNCOMPLICATED *FALCIPARUM* MALARIA: COMPARISON OF FIXED VERSUS CO-BLISTER FORMULATIONS

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Artesunate-Amodiaquine (AS-AQ) is the first line antimalarial treatment in 25 countries and until 2007 was available in a co-blistered, non fixed dose formulation. A fixed dose combination (FDC) of AS-AQ was introduced in 2007 to optimize the AS: AQ ratio and improve adherence. Current dosing recommendations for the FDC are according to three age ranges with 3 dosing strengths available. To assess the spectrum of total mg/kg dose of AQ administered and compare the effect on treatment efficacy of fixed and non fixed AS-AQ combinations, individual patient level data from clinical efficacy studies of uncomplicated *P. falciparum* were collated using standard algorithms. Factors associated with PCR adjusted efficacy were evaluated using Cox regression model with shared frailty to account for study effects. Data were available on 5410 patients from 24 studies conducted between 2003 and 2011 (5313 from Africa; 97 from Asia). 20 studies had 28 days follow up and 4 studies were followed up for 35 days or longer. 142 recrudescent cases were reported, the median time to recrudescence was 21 days [IQR: 21-28]. In the multivariate model risk factors for recrudescence were lower age categories categories [age < 1 year (AHR: 5.11, 95% CI: 1.30-20.06, P=0.0190), age 1-5 years (AHR: 6.53, 95% CI: 1.76-24.14, P=0.0049)], logged baseline parasitaemia (AHR: 1.17 [1.02-1.34], *P=0.0240*) and total mg/kg drug dosage received (AHR: 0.96, 95% CI: 0.93-0.99, P=0.0160). Adjusting for confounding factors the most significant risk factor for recrudescence was the use of non fixed dose formulation (AHR: 3.07 [1.51-6.21], *P=0.0021*). Patients treated with the FDC received a greater mg/kg dosage of AQ (median=29.56, IQR: 26.39-40.00) compared to those receiving a non FDC (median=25.00, IQR: 23.33-34.01).The fixed dose formulation provides better efficacy results than co-blisters probably related to improved dosages. Prospective comparative studies of ASAQ formulations are warranted to confirm the benefits on efficacy and effectiveness of fixed dose formulations and a higher target dose of AQ.

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PROBABILISTIC RECORD LINKAGE FOR MONITORING THE SAFETY OF ARTEMISININ BASED COMBINATION THERAPY IN THE FIRST TRIMESTER OF PREGNANCY IN RURAL SENEGAL

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There are insufficient data on the safety in early pregnancy of the artemisinin-type antimalarials. Assessing the risk of teratogenicity requires large sample sizes. Limited pharmacovigilance infrastructure exists in malaria-endemic countries. Monitoring drug safety in the first trimester is especially challenging as it requires prospective follow-up to reduce recall and survival biases and accurate assessment of gestational age. Record linkage approaches for pregnancy pharmacovigilance using routinely generated health records could be an efficient approach, but it has not been evaluated in resource-poor settings. The aim of this pilot study was to assess the feasibility of record linkage using routinely collected health care data as pragmatic means of monitoring the safety of artemisinin-based combination therapy (ACT) in early pregnancy in Senegal. Data (2004-2008) were extracted from paper-based registers from out-patient clinics, antenatal (ANC) and the delivery unit from a dispensary in rural Senegal and entered into databases. Probabilistic record linkage was used to identify pregnancies exposed to ACT in the first trimester of pregnancy. Two record linkage software packages (Link-Plus and FRIL) were compared and output data were reviewed independently by two investigators. Information on 685 pregnancies was extracted, 536 of which were eligible for record linkage; 95.3% of them resulted in live-births, 2.3% in stillbirths and 2.5% in miscarriages. Major congenital malformations were identified in 1.6% of births. Seventy-one and 75 matches between pregnancy outcome and the outpatient treatment registers were identified by both software packages. All the 7 pregnancies exposed to ACTs in the first trimester identified resulted in normal live-births. Probabilistic record linkage is a potential cost-effective method to assess the safety of

antimalarials in early pregnancy in resource-poor settings. It is suited to assess the risk of major birth defects and stillbirths in settings with good existing health records and well-defined target populations.

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EMPOWERING VILLAGE HEALTH TEAMS, A VALUE ADDITION TO HEALTH SERVICES DELIVERY IN RESOURCE LIMITED SETTINGS; CASE STUDY OF KIBOGA AND KYANKWANZI DISTRICTS IN UGANDA

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The majority of the people in Uganda, especially children, can not easily access health care, because of distant health facilities and a critical shortage of health workers. This has resulted in high mortality rates especially due to malaria (23%), pneumonia (21%), and diarrhoea (17%). Emerging evidence supports the unique role of community health workers referred to in Uganda as Village Health Teams (VHTs) play in providing first level health care in their communities. This paper provides a case study on how VHTs have increase health care provision for children under 5years for malaria, diarrhoea and respiratory tract infections in central Uganda. In the districts of Kiboga and Kyankwanzi, records of 4 government health facilities and VHTs attached to them were reviewed. Out Patient Department (OPD) attendance records and VHT data registers for children under 5 years between January to October for the years 2009 and 2011 was done. Data on malaria, diarrhoea and respiratory tract infections was analysed under four variables of OPD attendance, access to treatment, timeliness of treatment, and patient referral. Two third **(**2/3) of children with the three diseases accessed treatment and were healed in the communities by VHTs. Sixty four percent (64%) of malaria, 78% of diarrhoea and 65% of acute respiratory infections have been seen by VHTs. Only 12% of the children were referred to health facilities for further management. Furthermore 44% of all the children treated by VHTs, received their treatment within 24 hours of onset of illness. VHTs reduce attendance in OPD. This implies that there is reduced workload for the already constrained human resource at facility level. Timely treatment of diseases at community level is likely to reduce children that may slide into complications.

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A PILOT STUDY ON ANTI-MALARIAL INTERVENTIONS FOR MALARIA IN PREGNANCY IN EDO STATE, NIGERIA

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Malaria in pregnancy (MiP) is a major public health problem in Nigeria, despite available interventions. This abstract describes the collaborative efforts between (inter)national organizations, local communities, and stakeholders in the fight against MiP in Nigeria. In 2009, a study was undertaken in some communities in Edo State, Nigeria, assessing knowledge, attitude and practice (KAP) of MiP among women of reproductive age (15 - 49 yrs), and primary healthcare providers. Thereafter, interventions, including peer education on KAP of MiP were provided for health workers, and the women, through workshops, rallies, and door-to-door campaign. In addition, women advocacy groups were inaugurated and supported to continue the dissemination of appropriate KAP of MiP to all stakeholders in the communities. Finally, post-intervention survey was conducted to assess the impact of the intervention among the women. Furthermore, some factors responsible for low utilization of anti-malarial intervention during pregnancy were noted, including non-availability of insecticide treated bed nets (ITNs) and anti-malarial medications. A total of 1955 women of reproductive age (mean age ±sd, 27.88 ± 9.98) was surveyed. In all, 109 primary care providers (medical officers, nurses, community health extension workers, etc), and 37 women peer educators were trained. The flag-off awareness campaign attracted a large audience, via in-person and local television station. The door-to-door campaign and rallies reached about 3,000 persons within the study communities. Overall, peer education was effective in improving knowledge on malaria prevention among women of productive age, as knowledge increased significantly between pre- and post- intervention studies. Following the study, the National Malaria Control Program of Nigeria's Federal Ministry of Health, in 2011, through a local NGO, CHRADiP, freely distributed over 1000 long lasting ITNs to pregnant women and young children across the various communities in the state. The educational intervention improved KAP of MiP among the stakeholders in the communities. In addition, the primary care workers, and the entire community were mobilized and empowered on appropraite KAP of malaria. The provided free ITNs were appreciated. A national scale-up of a similar intervention is recommended.

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EFFICACY OF ARTESUNATE AND ARTESUNATE-AZITHROMYCIN FOR THE TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN VIETNAM

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Reports of reduced susceptibility of artesunate in the treatment of uncomplicated *Plasmodium falciparum* malaria in western Cambodia highlights the urgent need to contain and reduce the spread of artesunate resistant strains. As part of this effort it is important to monitor the spread artesunate resistance and to evaluate new artemisinin based combination therapies (ACT). The objective of the present study was to determine the efficacy of artesunate alone and artesunate-azithromycin for the treatment of uncomplicated *P. falciparum* malaria in south-central Vietnam. The latter ACT was assessed because of azithromycin's favourable pharmacokinetic properties and safety record in young children and pregnant women. In an open-labelled study carried out in 2010, 36 patients (children aged 6-14 years, n=10, adults aged 15-60 years, n=26) were allocated a 7-day course of artesunate (~4 mg/kg on D0, 2 mg/kg daily for D1 to D6) with a follow-up period of 28 days and 38 patients (children: n=14, adults: n=24) received a 3-day course of artesunate (4 mg/k daily) plus azithromycin (~20 mg/kg daily) with a follow-up period of 42 days. The treatments were well tolerated, with no obvious drug associated adverse events. The PCR genotype corrected cure rate was 91% for both treatment groups. This study showed that the malaria strains at the study site were still highly susceptible to artesunate alone. Artesunate-azithromycin was also efficacious in the treatment of *P. falciparum* malaria and may provide an alternative option for the treatment of young children and pregnant women.

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A SURVEILLANCE SYSTEM TO MONITOR THE QUALITY AND AUTHENTICITY OF ARTEMISININ COMBINATION TREATMENTS IN AFRICA AND SOUTHEAST ASIA

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Poor quality Artemisinin Combination Therapies (ACTs) in malaria-endemic countries pose an enormous threat to malaria patients. The lack of reliable estimates of the prevalence of poor quality ACTs and their impact on public health makes it difficult for the national regulatory authorities (NRAs) to determine the need and scale of interventions to put in place. Our aim is to provide robust estimates of the frequency of substandard, counterfeit and degraded artemisinin containing drugs, and to develop standardised methodologies for sample collection. As part of the overall project we have explored the use of different sampling strategies to collect drugs from public and private healthcare providers in Rwanda, Cambodia, Ghana and Tanzania, with sampling in other locations underway. Once collected all samples are logged onto a database, the packages scanned and, tablets weighed and measured. Qualitative (mass spectrometry, near infrared and Raman spectroscopy) and quantitative (high performance liquid chromatography and high performance liquid chromatography-mass spectrometry) content analyses are then conducted. Thus far over 3,500 ACTs have been analysed. Preliminary content analyses indicate that a number of samples fall below the internationally recommended thresholds (90-110 %) for their stated active pharmaceutical ingredient with variations found to occur both between and within batches of the same brand. To assist in classifying whether the ACTs are degraded, due to environmental impact rather than manufacturing practices, we are investigating the ageing of a set of patented ACTs in field and in laboratory based studies, with quantitative analysis carried out on these samples at regular intervals over a period of four years. Following cross verification between the three collaborating laboratories, the results will be shared with the country specific NRA and stored on the "Counterfeit Drug Forensic Network - CODFIN" database.

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LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP): A NOVEL TOOL TO INVESTIGATE MIXED MALARIA INFECTIONS IN MALARIA ELIMINATION SETTINGS

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Improved diagnostics for malaria will be required if elimination is to be achieved. We aimed to modify a novel nucleic acid amplification system, loop-mediated isothermal amplification (LAMP), to serve as a high-throughput, sensitive and specific diagnostic technique for the identification of sub-patent malaria infection. We developed a high-throughput 96-well plate LAMP (htLAMP) assay using a colorimetric agent that produces a visually detectable colour change. The htLAMP assay was applied to filter paper (FP) control parasitemia samples ranging from 0.0005- 2% to determine analytical sensitivity. HtLAMP was also applied to 98 whole blood (WB) samples from the Gambia and 25 FP samples from Ghana. Using primers to *Plasmodium falciparum* (htLAMP-Pf) and *Plasmodium* genus (htLAMP-Pg) on control samples the assay had a limit of detection of 0.0005% parasitemia (approximately 25 parasites/µL). Applied to WB samples from symptomatic children from the Gambia, htLAMP-Pg was positive in a sample containing 7.5 parasites/µL and, combined with htLAMP-Pf, correctly identified a *Plasmodium malariae* monoinfection.HtLAMP on FP samples from asymptomatic school children from Ghana identified the lowest parasitemia of 40 parasites/µL. The sensitivity of htLAMP-Pf and htLAMP-Pg compared with microscopy was 91% and 96% respectively and compared with nested PCR was 88% and 96% respectively.The performance of htLAMP has demonstrated good sensitivities when compared with microscopy and nested PCR for both whole blood and filter paper samples. Further optimization of the htLAMP assay would be required to achieve the desired analytical sensitivity of <10 parasites/µL.The high-throughput, colorimetric htLAMP assay shows promise as a diagnostic tool for rapid detection of low parasitemias encountered in elimination settings.

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DIAGNOSING MALARIA IN PREGNANCY: COMPARING IMMUNOFLUORESCENT MICROSCOPY TO OPTICAL MICROSCOPY AFTER GIEMSA STAINING

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Malaria in pregnancy remains a significant threat in sub-Saharan Africa as it is associated with sub-optimal pregnancy outcomes. The current standard of diagnosis, optical microscopy after staining with Giemsa, requires well trained microscopists and may require as long as two hours before results are obtained. Alternate rapid diagnostics tests thus need to be evaluated, particularly in pregnancy when changes in the immune response could potentially affect the performance of rapids tests based on antibody detection. We assessed the diagnostic performance of an alternate method, fluorescent microscopy, compared to optical microscopy after Giemsa staining.As part of baseline studies of mother-to-child transmission of HIV in the Buea Health District, 407 consenting pregnant women were enrolled. Venous blood samples were collected and tested by optical microscopy after Giemsa staining (OM) and by fluorescent microscopy (FM) using the Partec-Cyscope (Partec GmbH, Munster, Germany). All participants were asymptomatic at the time of enrollment. Both slides were read by experienced microscopists and evaluated qualitatively as being positive or negative for plasmodia.Of the 407 samples tested by OM, 255 (62.5%) were plasmodium-positive. Of these 255, 207 were also plasmodium-positive by FM, thus a sensitivity of 81.1% (95%CI: 75.8, 85.8%). Of the 152 samples negative by OM, 75 were also negative by FM, thus a specificity of 49.3% (95%CI: 41.1, 57.6%). The positive and negative predictive values of FM were respectively 72.9% (95%CI: 67.3, 78.0%) and 61.0% (95%CI: 51.8, 69.6%). The percentage agreement between both methods was 69.3% (Kappa=0.32, p-value<0.01). There was moderate agreement between FM and OM. The low specificity and negative predictive value of FM suggest a high likelihood of false negative results if FM is used in place of OM.

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THERMAL CONTRAST SIGNIFICANTLY IMPROVES THE SENSITIVITY OF LATERAL FLOW ASSAYS FOR MALARIA DIAGNOSIS

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Malaria rapid diagnostic tests (RDTs) using lateral flow immunoassays (LFAs) are one of the few low-cost assays that can diagnose malaria in a point of care setting without laboratory infrastructure. With LFAs, a positive detection occurs when the test region of the assay membrane strip appears visibly red if the target analyte from a patient's sample is captured by an antibody bound to the membrane and an antibody bound

to the surface of gold nanoparticles. This sandwich capture occurs as antigen-bound gold nanoparticles migrate across the membrane to form the test line. When compared to microscopy, the best of the current LFAs have >95% sensitivity and specificity for *Plasmodium falciparum* infections in which parasitemia is ≥200 parasites/μL, but have significantly decreased sensitivity for infections with <200 parasites/μL and for *P. vivax, P. ovale* and *P. malariae*. Malaria elimination campaigns will require RDTs with a sensitivity that exceeds that of microscopy. We recently demonstrated that the sensitivity of RDTs can be dramatically enhanced by laser heating of the gold nanoparticles resulting in quantifiable release of heat. An infrared camera can then measure the heat released, which is directly proportional to the number of gold nanoparticles, and this technique can quantitatively measure the antigen burden in the sample. This new technology is termed thermal contrast and was recently demonstrated for Cryptococcal meningitis, as reported previously. Herein we show that this technology can also be used to enhance the sensitivity of RDTs for malaria, showing an 8-fold increase in sensitivity as compared to standard RDT testing during serial dilutions of clinically positive *P. falciparum* blood samples. Thus, while current RDTs have a limit of detection at 200 parasites/μL, thermal contrast can enable detection at the level of ~25 parasites/μL, significantly improving the sensitivity of RDTs to malaria in those with low-level parasitemia. Further improvement on the order of 100-fold in sensitivity is possible with redesign of LFAs to reduce nonspecific background laser absorption and enhance specific gold nanoparticle absorption. Finally, inexpensive existing technologies are being evaluated to design a robust, battery operated point-of-care RDT thermal contrast reader (<$100) for resource limited settings.

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PERSPECTIVES ON MALARIA RAPID DIAGNOSTIC TESTS AFTER NATIONAL ROLLOUT IN TANZANIA'S PUBLIC SECTOR - PROVIDER AND CONSUMER VIEWS FROM MBEYA REGION

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As part of its national strategy to improve malaria case management, Tanzania has been gradually rolling out malaria rapid diagnostic tests (mRDT) in the public sector since 2008. A multidisciplinary evaluation to assess the effectiveness of this strategy is currently underway. We present results of qualitative research conducted in two districts in Mbeya Region where mRDTs were introduced in February 2011. Qualitative interviews were conducted with health authorities, providers and community members about their experiences with malaria diagnosis and treatment post mRDT implementation. A total of 28 interview transcripts were reviewed for content analysis. Several conflicting views and practices with respect to mRDTs emerged. Whereas laboratory and pharmacy officers were more likely to express confidence in the accuracy of mRDTs, other health authorities were less convinced of their usefulness as a diagnostic tool; some suggested more studies to assess the quality of mRDTs were needed. Others expressed concerns that clinicians were ignoring negative mRDTs in favor of artemether-lumefantrine (ALu) treatment. Our interviews with providers and community members confirm their suspicions. While some providers acknowledged ignoring negative mRDTs results for patients with malaria symptoms, they also noted that such patients often improved after ALu treatment. Other providers adopted a "wait and see" approach, advising their patients to return in 2-3 days if symptoms persisted. Although the extent of such practices is not known, the use of ALu for negative mRDTs was cited as one of the malaria challenges for the region. Stock outs of mRDTs were mentioned as another. Regional authorities noted that within the first 8 months of implementation, 4 out of 8 districts had experienced a stock out. Although mRDT stock outs were less disruptive for facilities that also practiced microscopy, facilities without microscopes reported reverting to clinical diagnosis. These challenges will need to be addressed early on if improvements in malaria case management are to be achieved. Strategies to consider include identifying effective measures to improve provider adherence to mRDTs, as well as addressing bottlenecks in the mRDT supply chain.

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STUDY OF HOSPITAL BASED MALARIA CASES IN THE PEDIATRIC DEPARTMENT OF KORLE BU TEACHING HOSPITAL, GHANA

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Malaria kills about one million children, under five years of age, each year worldwide, with nine out of 10 deaths occurring in sub-Saharan Africa. This study was carried out to determine the incidence of malaria in the pediatric department of Korle Bu Teaching Hospital from January 2011 to October 2011, and to compare available diagnostic tests for malaria. 978 suspected cases of malaria (507 males and 471 females, aged 1 day - 12 years), attending the OPD and admitted as inpatients in the ER of the Pediatric Department were included in this study. 1.0 mls of blood sample was collected into EDTA bottle. Thick and thin smears were prepared, stained and examined. Subsequently, the blood samples were subjected to antigen detection using the First Response Malaria pLDH/ HRP 2 Combo Test according to the manufacturer's instructions. The results were tabulated and analyzed statistically. 51 cases out of 978 suspected cases were positive for malaria, with an incidence of 5.2%. Out of these 40 (78.4%) were positive for *Plasmodium falciparum*, 5 (9.8%) were positive for *P. malariae*, 2 (3.9%) were positive for *P. ovale*, and 4 (7.8%) were positive for both *P. falciparum* and *P. malariae*. The First Response Malaria pLDH/ HRP 2 Combo Test detected 51 positive cases compared with the blood smear study, which detected 41 cases. 36 cases were detected both by the First Response Malaria pLDH/ HRP 2 Combo Test and blood smear study. 15 cases were positive by the First Response Malaria pLDH/ HRP 2 Combo Test, but not by the blood smear study. 5 cases detected to be positive by the blood smear study were found to be negative by the First Response Malaria pLDH/ HRP 2 Combo Test. 937 cases were negative both by the First Response Malaria pLDH/ HRP 2 Combo Test and the blood smear study. Among 51 positive cases, 35 were males with a percentage of 68.6% as compared to females (31.4%).The sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic efficiency of the First Response Malaria pLDH/ HRP 2 Combo Test when compared to microscopy, were 87.5, 96.8,90, 98.9, and 96%, respectively. In conclusion, the incidence of malaria in this present study was 5.2%. The sensitivity of First Response Malaria pLDH/ HRP 2 Combo Test is very close to microscopy. It is a simple, sensitive, and effective diagnostic test for *P. falciparum, P. malariae, P. vivax* and *P. ovale* malaria.

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ADDRESSING OVER- AND UNDER-DIAGNOSIS OF MALARIA IN TANZANIA: AN EVALUATION OF LARGE-SCALE IMPLEMENTATION OF MALARIA RAPID DIAGNOSTIC TESTS (MRDTS) IN THREE REGIONS WITH VARYING MALARIA EPIDEMIOLOGY

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Artemisinin based combination therapy (ACT) is the first line drug in most malaria-endemic countries, but there are concerns that quality of care remains poor. Patients needing ACT often do not receive it, but there is also considerable over-treatment due to the lack of accurate diagnosis and inappropriate management. In 2010-2012, Tanzania rolled-out malaria rapid diagnostic tests (mRDTs) at government health facilities to improve treatment of febrile illness. Here, we report results of health facility surveys to assess treatment practices before and after mRDT scale up in three regions with varying malaria epidemiology. Patients with fever in the previous 48 hours were enrolled at 320 randomly selected health facilities in Mwanza, Mbeya, and Mtwara regions in May - October 2010 and March - August 2012. Patients were interviewed following their consultation, and data were collected on patient characteristics, previous treatment for fever, and care received at the facility. Finger prick blood samples were taken by study staff to test for malaria parasitemia. Health workers seeing patients were also interviewed about their training and supervision, knowledge, and facility stocks of antimalarials and mRDTs. At baseline, data were collected on 1746 patients, of which only 15.9% received a diagnostic test from facility health workers. Based on study blood smears, 20.9% tested positive in Mtwara, 6.6% in Mwanza, and 1.6% in Mbeya. An ACT was obtained by 65.8% of patients testing positive by the study blood slide and 39.0% of patients testing negative, meaning that overall only 58.5% of patients received appropriate malaria treatment given their study blood smear result. We will compare these results with those from 2012 to evaluate the success of mRDT roll-out at addressing over- and under-diagnosis of malaria, and the role of stock-outs and health worker practices in addressing these key problems. These data will contribute to enhancing interventions to increase appropriate treatment of patients with and without malaria in Tanzania and other malaria-endemic countries.

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ACCURACY OF TWO RAPID DIAGNOSTIC TESTS FOR DIAGNOSIS AND MONITORING TREATMENT OF MALARIA IN A HIGH TRANSMISSION SETTING IN UGANDA

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Rapid diagnostic tests (RDTs) for malaria are simple to perform and may improve fever management in malaria endemic areas where microscopy is not readily available. Most RDTs detect Histidine- rich protein 2 (HRP2) which persists in the blood stream for variable lengths of time after treatment. *Plasmodium* lactate dehydrogenase (pLDH) based RDTs become negative more quickly. We assessed the accuracy of HRP2 and pLDH based malaria RDTs for initial diagnosis of uncomplicated malaria, treatment monitoring and diagnosis of recurrent episodes of malaria in young children in a hyperendemic area in Uganda. Accuracy of the RDTs for diagnosis of malaria was compared using expert microscopy as a gold standard in 308 episodes of fever in a cohort of children under five years. We followed up 131 children with microscopically confirmed malaria at 7 day intervals for 28 days to determine clearance time of HRP2 and pLDH antigenemia. Both RDTs were done for children with recurrent episodes of fever after treatment of malaria. Of the 308 children tested, sensitivity was 98% for HRP2 and 87.1% for pLDH; specificity was 54.7% for HRP2 and 95.3% for pLDH. Positive predictive value (PPV) was 80.5% for HRP2 and 97.2% for pLDH; negative predictive value (NPV) was 93.5% for HRP2 and 79.5% for pLDH. Persistent antigenemia from recent malaria episodes contributed to the low specificity of HRP2 while the lower sensitivity of pLDH was due to poor antigen detection at low parasite densities. The mean duration of antigenemia was 21 days for HRP2 and 2 days for pLDH. Pre-treatment parasite density predicted the duration of antigenemia of HRP2. We documented 61 episodes of fever after antimalarial treatment. For these, HRP2 had sensitivity of 100%, specificity of 34.5%, PPV of 62.7% and NPV of 100%. pDLH had sensitivity of 90.6%, specificity of 100%, PPV of 100% and NPV of 90.6%. The HRP2 based RDT though accurate for initial diagnosis of malaria, was limited by low specificity due to persistent antigenemia.The pLDH based RDT showed rapid clearance of antigenemia and was accurate for diagnosis of malaria in children with recurrent fever after treatment. In patients who have had malaria in the previous three weeks and present with fever, pLDH based RDTs should be used to monitor treatment and diagnose new episodes of malaria.

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THE DIAGNOSTIC VALUES OF RAPID DIAGNOSTIC TEST BASED ON HISTINE RICH PROTEIN II OF CARESTARTTM MALARIA IN DETECTING MALARIA INFECTION IN PREGNANT WOMEN SAN AND KITA

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We conducted from July 2009 to February 2010 in San and Kita (Mali), a study on the diagnostic values of the Histine Rich Protein II (HRP-II) CareStartTM Malaria compared to the thick smear for the detection of malaria infection during pregnancy as part of a pilot study for monitoring the therapeutic efficacy of Sulfadoxine-Pyrimethamine during *in vivo* testing of 42 days. Result showed both the sensitivity and specificity of 95%. Positive and negative predictive values were of 86% and 98% respectively, and a kappa value was 0.87. There were 5% of false positives and negatives.When tracking *in vivo*, the proportion of positive HRP-II CareStartTM was higher than that observed in the thick film. CareStartTM Malaria positivity is proportional to parasite density detected by the thick film. HRP-II of CareStartTM Malaria is simple to use and fast. The HRP-II TDR can be used in the detection of malaria infection among pregnant women.

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EVALUATION OF PRE- AND POST-TRAINING KNOWLEDGE AND PRACTICES OF HEALTH WORKERS IN THE USE OF RAPID DIAGNOSTIC TEST FOR PARASITOLOGICAL DIAGNOSIS OF UNCOMPLICATED MALARIA IN CAMEROON

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Rapid diagnostic tests (RDTs) for malaria diagnosis have attracted interest in recent years because of their high specificity and sensitivity and are suitable for resource-constrained settings as they require minimal infrastructure. However, many health workers who are the main players for the effective use of this new technique do not yet master it. The Cameroon National Malaria Control Programme (NMCP) only recently

introduced RDTs in 50 pilot districts in 2011. Health workers (HWs) from mission and public health facilities in Yaounde and Bamenda cluster randomized in a research to evaluate the provision of appropriate treatment to malaria patients were invited to attend a 1 day and 3 days workshop on "Ensuring appropriate treatment for uncomplicated malaria" and "Improving quality of care for management of suspected malaria" respectively. All workshop attendees completed a pre training questionnaire which covered aspects such as clinical manifestation and methods of malaria diagnosis, the role of an RDT, who should conduct an RDT, the practical steps, time to read and interpretation of the results, treatment according to test results. During the training, HWs received lectures and practical exercises on all the above mentioned aspects including practical steps with assistance of a 16- step WHO RDT job aid and treatment guidelines from NMCP. Participants were also individually supervised during the performance of an RDT and graded using a checklist. The same questionnaire was used for post training evaluation. Of the 54 HWs from Yaounde, 62.5% were nurses, 20.8% medical doctors and 16.7% laboratory technicians. The knowledge increase on clinical manifestation and diagnostic methods for malaria for pre and post training was 10.42% while knowledge on RDT use had an increase of 52.3%. Knowledge on treatment based on test results had an increase of 28.2% while practical skills improved from 0% to 80%. Of the 40 HWs from Bamenda, 62.5% were nurses, 25% medical doctors and 12.5% laboratory technicians. The knowledge increase on clinical manifestation and diagnostic methods for pre and post training was 8.9% while knowledge on the RDT had a 35.4% increase. The knowledge on treatment based on test results had an increase of 17.6% while practical skills improved from 0% to 86%. If HWs are given appropriate training, clear instructions with appropriate job aids, they can use RDTs appropriately irrespective of their cadre and setting.

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MALARIA PARASITE DENSITY ESTIMATED FROM ACTUAL WBC COUNT OF PATIENTS CORRELATES WITH ESTABLISHED WBC REFERENCE VALUE IN CENTRAL GHANA

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White Blood Cells count (WBCc) is a bed-rock in the estimation of malaria parasite density in malaria field trials, interventions and patient management. WBCs are indirectly and relatively used in microscopy to estimate the density of malaria parasite infections. Due to frequent lack of facilities, in some malaria endemic countries, to quantify WBCc of patients, an assumed WBCc of 8.0 X 109/L has been set by the WHO to help in estimating malaria parasite densities. The comparative analysis study, in Central Ghana, compiled laboratory data of 5902 *Plasmodium falciparum* (Pf) malaria parasite positive samples. Samples were obtained from consented participants of age-group less than 5 years. Full Blood Counts (FBC) of participants' samples were analysed using the ABX Micros 60 Haematology Analyzer. Blood slides were read by two competent microscopists to produce concordant results. All internal and external quality control measures were carried out appropriately. Parasite densities were calculated using participants' absolute WBCc and assumed WBCc of 5,000 to 10,000 per microlitre of blood. From the 5902 Pf malaria positive samples, the mean (SD) WBCc and geometric mean parasite density were 10.4 (4.6) × 109/L and 7557/µL (95% CI 7144/µL to 7994/µL) respectively. The difference in the geometric mean parasite densities calculated using absolute WBCs and compared to densities with assumed WBCs counts were significantly lower for 5.0 × 109/L; 3937/µL, 6.0 × 109/L; 4725/µL and 8.0 × 109/L; 6300/µL. However, the difference in geometric mean parasite density, 7874/µL (95% CI, 7445/µL to 8328/µL), with assumed WBCc of 10.0 × 109/L was not significant. In conclusion, using the assumed WBCc to estimate malaria parasite densities in Pf infected children less than 5years could result in significant errors in the estimation of parasite burden in a malaria endemic region. Assumed WBCc of 10.0 × 109/L at 95% CI of geometric mean of parasite density statistically agreed with the parasite densities produce by the absolute WBCc of participants. This correlates with the established reference value for WBC in Central Ghana. We therefore suggest where available, the use of the WBC reference value to estimate malaria parasite density when obtaining absolute WBCc is not possible especially in drug efficacy and vaccine trials.

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PRODUCTION OF VERTICAL FLOW RAPID MALARIA TEST KIT TO DETECT *PLASMODIUM FALCIPARUM* AND *PLASMODIUM VIVAX*

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An endeavour to produce in-house rapid diagnostic test in Thailand to supplement the use of various commercial test kits available the market. The test aimed to produce a vertical flow immunoclomatographic test to detect either *Plasmodium falciparum* or PAN malaria parasites and hoped to be the alternative tool for field users. The test were produced by using the in-house monoclonal antibodies produced against either *Plasmodium* lactate dehydrogenase (pLDH) or *Plasmodium* glyceraldehydes-3-phosphate dehydrogenase (pGAPDH). Preliminary study was done against 38 wild type malaria and 39 negative control samples and found that the test kit gave sensitivity and specificity to *P. falciparum* 89.5 % and 98.3 % and to *P. vivax* 82.4 % and 98.8 % respectively. Even the test gave somewhat high diagnostic values but its sensitivity positive correlated with parasitemia levels. However, at least, this study got an alternative RDT prototype to be validated its feasibility for using in field onward.

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INTRODUCING RAPID DIAGNOSTIC TESTS INTO COMMUNITY-BASED MANAGEMENT OF MALARIA: EVIDENCE FROM A CLUSTER-RANDOMIZED TRIAL IN TWO AREAS OF HIGH AND LOW TRANSMISSION IN UGANDA

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Universal access to diagnostic testing for malaria is now recommended by WHO, to encompass all levels of health care, including community-based treatment programmes. Rapid diagnostic tests (RDTs) provide a simple means of confirming malaria diagnosis in locations lacking electricity and qualified health staff. Some countries have begun to introduce RDTs at community level, and data on the impact of diagnostic testing on treatment and referral practices by community health workers is still limited. A cluster-randomised trial to evaluate the impact and cost-effectiveness of RDTs when used by community medicine distributors (CMDs), compared with presumptive treatment, has been conducted in two areas with contrasting malaria transmission in Rukungiri District, Uganda since June 2010. The trial aims to evaluate the impact of diagnostic testing on the proportion of children who receive appropriate ACT treatment and referral under low and high transmission, as defined by malaria microscopy on a research slide collected at the same time as the RDT. The study will also provide evidence on the operational challenges and community acceptability of RDTs. A total of 120 communities (379 CMDs) were randomised to training either in use of RDTs or presumptive

diagnosis of malaria. All CMDs were trained on how to give antimalarial treatment with ACTs, rectal artesunate pre-referral treatment, and when to refer. Supporting interventions included activities to raise community awareness, and close support supervision to CMDs for the first six months of implementation. Since January 2011, supervision has been scaled back to mimic levels typically seen in health systems in rural Africa. Nonetheless, adherence to RDT results by CMDs has remained high, with over 95% of ACT treatments given being consistent with the results of the RDT test. We will present data on adherence to RDT result and treatment guidelines by CMDs; compare referral practices and frequency of patients following through with referral in the two arms; and changes in these outcomes, over the first 18 months of the trial.

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INTRODUCING RAPID DIAGNOSTIC TESTING FOR MALARIA INTO THE PRIVATE SECTOR: EVIDENCE FROM A CLUSTER-RANDOMIZED TRIAL IN REGISTERED DRUG SHOPS IN UGANDA

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Universal access to diagnostic testing for malaria is now recommended by WHO, to encompass all treatment providers. Many malaria cases are treated outside the formal health sector, with drug shops often being the first, and only, source of treatment. Rapid diagnostic tests (RDTs) provide a simple means of confirming malaria diagnosis in drug shops, and improved diagnosis may also help to ensure that the drugs sold are appropriate. As yet, there is little evidence of the impact of diagnostic testing on antimalarial drug sales and referral practices by drug shops, particularly in Africa. A cluster-randomised trial to evaluate the impact and cost-effectiveness of using RDTs in registered drug shops, compared with presumptive treatment, has been conducted in Mukono District, Uganda since October 2010. The trial aims to evaluate the impact of diagnostic testing on the proportion of drug shop clients who receive appropriate ACT treatment, in line with parasitological status as defined by malaria microscopy on a research slide collected at the same time as the RDT. The study will also provide evidence on the feasibility; operational challenges and acceptability of this approach. A total of 60 drug shops were randomised to receive training either in the use of RDTs or presumptive diagnosis of malaria. All drug shop vendors (DSVs) were trained on the national malaria treatment guidelines, use of rectal artesunate pre-referral treatment, and when to refer. Supporting interventions included activities to raise community awareness, and close support supervision to DSVs for the first 3 months of implementation. Since January 2011, supervision has been scaled back to mimic levels typically seen in health systems in rural Africa. Nonetheless, adherence to RDT results by DSVs has remained high, with over 95% of ACT treatments sold being consistent with RDT test results. We will describe the design of the intervention in drug shops, and present data on adherence to RDT result and treatment guidelines by DSVs; referral practices; and changes over the first 15 months of the trial.

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SCALING-UP RAPID DIAGNOSTIC TESTS FOR MALARIA: BARRIERS AND OPPORTUNITIES IN NIGERIA'S PRIVATE SECTOR

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Throughout Nigeria, about 80% of treatment for fevers occurs in private shops, pharmacies and clinics. Many private practitioners presumptively diagnose childhood fevers and a multitude of other symptoms as malaria without conducting blood tests, for a variety of reasons. With the increased availability of artemisinin combination therapy (ACTs) to effectively treat malaria, there is growing recognition that accurate diagnosis prior to treatment is needed. Effective diagnosis not only saves on health expenses and reduces the risk of drug resistance, but also reduces the risk of misdiagnosis and delayed treatment of non-malaria illness. The Global Health Group at UCSF conducted qualitative research in early 2012 in collaboration with the University of Ibadan, Nigeria, to determine the availability and acceptability of using Rapid Diagnostic Tests (RDTs) as an easy and inexpensive tool for diagnosis of malaria in the absence of microscopy. Results from qualatative analysis indicate very little knowledge or use of RDTs and that significant structural barriers exist in deploying RDTs within the private sector. These barriers include legal and technical constraints among different types of providers; regulatory limitations; widespread beliefs that diagnostic tests are not needed and that results are unreliable; and inadequate incentive structures for balancing profit motives with priorities for providing quality care. This research suggests that private sector providers may respond to patient requests for diagnosis prior to treatment if awareness of the value of diagnosis is increased and greater demand for RDTs can be fostered. A second phase of research is underway which will test malaria prevalence as well as acceptability and adherence to RDT results among purchasers of anti-malaria medication at private outlets in Nigeria.

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HIGH FREQUENCY MALARIA PARASITE DETECTION BY BUFFY-COAT SMEAR AMONG PATIENTS WITH NEGATIVE THICK BLOOD FILM TESTS

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Malaria remains one of the world's most serious global health concerns. Prompt and accurate diagnosis is critical to effective management of malaria. Although microscopy by thin- and thick-blood smears is the current standard for malaria diagnosis, there is a risk of false negative results when the patient has recently been treated with antimalarial medications. To explore how frequently *falciparum* malaria parasites are detected, by quantitative buffy-coat capillary tube test, among patients with prior negative results by thick-film blood-smear tests. We studied 36 patients with uncomplicated *falciparum* malaria, confirmed by conventional thick- and thin-film microscopy diagnostic methods. The patients were admitted and treated at the Bangkok Hospital for Tropical Diseases. Fingerpricks for conventional blood smear and concurrent buffy coat blood smears were conducted every 6 hours until the patients exhibited negative parasitemia, and then finger-prick tests were performed daily until each patient was discharged from hospital. A negative result by conventional thick-film test was compared with the concurrent buffy-coat thick-film test. By study method, we found that of 36 patients with negative thick films, buffy-coat smears detected 10 patients (27.8%) with asexual form *Plasmodium falciparum.* We concluded that quantitative buffy coat capillary tube tests can accurately and efficiently detect malaria parasites, even when conventional thick films show negative parasitemia.

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COMPARATIVE EFFICACY OF UNCONTROLLED AND CONTROLLED INTERMITTENT PREVENTIVE TREATMENT DURING PREGNANCY (IPTP) WITH COMBINED USED OF LLTNS IN HIGH RESISTANCE AREA TO SULFADOXINE-PYRIMETHAMINE IN CÔTE D'IVOIRE

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In recent years, Intermittent Preventive Treatment for pregnancy (IPTp) with SP has become policy in much of sub-Saharan Africa. But resistance to SP has been spreading across sub-Saharan Africa and thus the effectiveness of SP-IPT has been questioned. The present study, therefore, sought to assess incidence of placental malaria, LBW and aneamia of two approaches IPTp-SP (DOT scheme versus no DOT) in Anonkoua-kouté and Samo where the reported prevalence of *dfr* single mutant 108 was respectively 62% and 52,2%. The study was a longitudinal design involving pregnant women and was conducted in Anonkoua-kouté (Côte d'Ivoire), a suburban area, and Samo, a rural area, from January 2008 through March 2009. Women of a pregnancy less than 28 weeks duration were randomized to receive SP (1500 mg of sulfadoxine and 75 mg of pyrimethamine) in a single intake twice and were followed up monthly until delivery. Doses were administered under supervision in the controlled IPTp group, while in the uncontrolled IPTp group, drug was given free to women and it was recommended to take it at home. The primary end point was the proportion of low birth-weight (LBW) infants (body weight <2500 g) and the secondary, the rate of severe anaemia and placental malaria detected at delivery. A total of 420 pregnant women were enrolled (212 and 208 respectively in controlled and uncontrolled groups). Delivery outcome was available for 378 women. In the modified intention to treat (ITT) analysis, LBW infants were born from 15.5% of women of the uncontrolled IPTp group and from 11.9% of women on controlled IPTp group (p= 0.31). The per-protocol population (PP) analysis showed consistent results. The proportions of women with placental malaria infection, moderate anemia (Hb<11 g/dL), and severe anemia (Hb <8 g/dL) at delivery were similar between the two groups (p>0.05). In conclusion, the study showed that the two approaches were equivalent suggesting the use of unsupervised IPTp with SP free of charge in areas where implementation of DOT scheme suffer from many constraints.

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EFFICACY OF ARTEMETHER/LUMEFANTRINE SINCE ADOPTED AS A FIRST LINE TREATMENT FOR UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN ETHIOPIA IN 2004

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In Ethiopia, unacceptably high level of resistance to sulphadoxine/pyrimethamine prompted the change to a combination of artemether and lumefantrine (AL) as a national first line treatment for uncomplicated *Plasmodium falciparum* malaria in 2004. Regular monitoring of the efficacy of the recommended regimen for *falciparum* malaria is essential to suggest whether the required high level of efficacy is maintained or to detect any early indication of resistance. These studies were conducted to assess the current level of AL efficacy in the country and provide credible information to national malaria control program managers for evidence based decision making. The studies were conducted between 2007-2011 malaria peak transmission seasons in seven sentinel sites using the revised WHO protocol. A minimum of ninety patients with uncomplicated *P. falciparum* malaria aged six months and above were enrolled in each study site. Each patient was treated with a standard six dose regimen of AL given twice daily for three days under partial supervision. The clinical and parasitological responses were assessed during a twenty eight days follow up period. Outcome of treatment were defined according to the standard WHO classification. Recurrent parasitaemia were genotyped to distinguish between recrudescence and new infection. PCR corrected adequate clinical and parasitological response (ACRP) at Day twenty eight in the per protocol analysis was greater than 95% in all sites except in Shele where the ACRP was 92.5%. There was no early treatment failure and most of the recurrent infections were due to late parasitological failure. Parasite and fever clearance rates were rapid and all patients were cleared of their gametocytes by day 14. Mean hemoglobin value had also improved on day twenty eight compared with the baseline. No serious adverse events were reported. However, mouth ulcer was recorded in some children after treatment and resolved spontaneously. A regimen of AL is highly effective in the study localities, after six years of use as first line treatment in the country. The high cure rate of AL reported in this study is encouraging and support the continued use as first line treatment for uncomplicated *falciparum* malaria in the country. However the 7.5% recrudescence infections observed in Shele highlights the need for regular monitoring the efficacy in Shele and other part of the country.

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ASSESSMENT OF THE MOLECULAR MARKER OF *PLASMODIUM FALCIPARUM* CHLOROQUINE RESISTANCE (PFCRT) IN SENEGAL AFTER SEVERAL YEARS OF CHLOROQUINE WITHDRAWAL

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Since 2006, the artemisinin- combination therapies (ACTs); artemether–lumefantrine (AL) and artesunate plus amodiaquine (AS/AQ) were adopted for uncomplicated malaria treatment. After several years of CQ withdrawal the current study wished to determine the level of CQ resistance at the molecular level in selected sites in Senegal since interest in using CQ again has been raised by the scientific community. Finger prick blood samples were collected from *Plasmodium falciparum* positive children below the age of 10 (n = 474) during cross sectional surveys conducted in two study sites in Senegal with different malaria transmission level. All samples were analyzed for single nucleotide polymorphisms (SNPs) in the *P. falciparum* chloroquine resistance transporter gene (*Pfcrt* – codons 72-76) using PCR-SSOP ELISA and Real Time-PCR methods. In total, 449 blood samples (94.7%) were PCR positive, 285 and 164 from Central and Southern sites of Senegal, respectively. In both study areas the prevalence of the *Pfcrt* wild type single CVMNK haplotype was very high; Central study at 70.5% in 2009 and 74.8% in 2010 and in Southern study site at 65.4% in 2010 and 71.0% in 2011. Comparing data with older studies in Senegal, a sharp decline of the mutant type *Pfcrt* prevalence is evident. From 65%, 64% and 59.5% in samples collected from various sites in 2000, 2001 and 2004, to approximately 30% in our study. A similar decrease in mutant type prevalence is noted in other neighboring countries. With the continued development of increased CQ susceptibility in many African countries it may be possible to re-introduce CQ again in the near future, in a drug combination, possibly given to non-vulnerable groups and demanding a close monitoring of possible reemergence of CQ resistance development.

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MONITORING ANTIMALARIAL DRUG RESPONSE IN *PLASMODIUM FALCIPARUM* FIELD ISOLATES USING AN *EX VIVO* DAPI ASSAY

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Malaria treatment efforts are hindered by the rapid emergence and spread of drug resistant parasites. Simple assays to monitor parasite drug response in clinical samples are important, as they can detect drug resistance before it becomes clinically apparent as well as inform changes in treatment policy to help prevent the spread of resistant parasites. We surveyed malaria cases in a clinic in Thiés, Senegal from 2008-2011 and employed a DAPI-based *ex vivo* drug assay to test parasite response to amodiaquine, chloroquine and mefloquine in approximately 400 clinical isolates. We genotyped known drug resistance-associated mutations and culture adapted a subset of parasites in order to compare their *in vitro* and *ex vivo* drug responses. The DAPI *ex vivo* drug assay is comparable or superior to SYBR-based assays using clinical samples, with a median signal to noise ratio of 4:1 and excellent agreement between technical replicates. Mutations in *pfcrt* and *pfmdr1* were associated with changes in drug response, and we observed strong concordance between the *ex vivo* and *in vitro* IC_{50}s of culture adapted parasites. Overall, the DAPI *ex vivo* assay is robust and can be used to monitor parasite response to antimalarial drugs in field settings.

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SNPS ON ABC TRANSPORTERS AND *IN VIVO* MALARIA PARASITE NON CLEARANCE AFTER CHLOROQUINE TREATMENT IN MALIAN CHILDREN

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Plasmodium falciparum malaria remains one of the major causes of morbidity and mortality in sub-Saharan Africa. PfCRT K76T mutation was demonstrated to play a central role in the *P. falciparum* resistance to chloroquine. Previous study have shown that SNPs on several ABC transporters genes are associated with *in vitro* chloroquine resistance. We aimed to find any association between SNPs on ABC transporter and the *in vivo* parasite non clearance after chloroquine treatment in Mali. We carried out a chloroquine efficacy study in the rural village of Kollé, Mali. P *falciparum* DNA was extracted from filter paper and SNPs on Pfcrt, Pfmdr1, PfG30 and PfG47 were analyzed by nested and MS PCR.The study protocol and informed consent document were reviewed and approved by the National ethical committee.The data were statistically analysed by Epi Info® and STATA. 196 children suffering from uncomplicated malaria were included and 54 (27.5%)of them failed to the treatment at D14. The mutant alleles Pfcrt 76T and Pfmdr1 86Y were associated with parasite non clearance with p=0.00001 and 0.03 respectively. However, the association of SNPs on PfG30 and PfG47 genes with parasite non clearance was not statistically significant, p =0.43 and 0.57 respectively. The logistical regression analysis showed that the mutant allele Pfmdr186Y contributed positively to the Pfcrt 76T parasites non clearance (p=0.02). This gene has been already described as a modulator of chloroquine resistance in several *in vitro* and *in vivo* studies from different settings. However, the SNPs on PfG30 and PfG47 genes did not contribute to the parasite non clearance. In conclusion, our findings have shown a lack of association between SNPs on the new putative transporters genes and parasite non clearance in children in Mali. But Pfcrt76T and Pfmdr186Y alleles were associated with the *in vivo* parasite non clearance in these settings.

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PREVALENCE OF MUTATION OF PFCRT AFTER THE USE OF AMODIAQUINE IN INTERMITTENT PREVENTIVE TREATMENT IN CHILDREN (IPTC) IN SENEGAL

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Chloroquine was until 2002 the most commonly drug used against uncomplicated malaria in Africa in general and Senegal in particular. After several years of the withdrawal of chloroquine due to an high level of resistance of *Plasmodium falciparum*. Studies were conducted in Africa and showed a decline in the prevalence of resistance marker pfcrt-76T, molecular marker of *P. falciparum* resistance to chloroquine and amodiaquine. In Senegal the IPTc is a strategy for proventing malaria in children using sulfadoxine-pyrimethamine and amodiaquine. So the aim of this study is to evaluate the prevalence of pfcrt mutation in Senegal after three years of implementation of IPTc. This study was conducted in three health districts in Senegal (Mbour, Fatick and Bambey) with 54 health posts. The genotype of the pfcrt gene for polymorphisms C72S and K76T was determined in at least two multiplex real-time PCR runs with full agreements using the Rotorgene 3000 platform representing CVIET, CVMNK and SVMNT haplotypes. 3D7, Dd2 and 7G8 DNA obtained from the Malaria Research Reagent Resource (MR4) was used to provide sequence-specific positive control and nuclease free water was included as a negative control. Analysis was done with 47 isolates in 2008, all 2009 samples (n= 42) and 116 of the 125 isolates in 2010, were PCR positive. 10 (21%), 21 (48%) and 48 (41%) carried the CVIET haplotypes in 2008, 2009 and 2010 respectively. The SVMNK haplotypes were found in 32 (68%), 18 (42%) and 58 (50%) isolates in 2008, 2009 and 2010 respectively. Mixed haplotypes infections (CVMNK/CVIET) were found in 5 (10%), 3 (6%) and 10 (8%) of the 2008, 2009 and 2010 samples respectively. The SVMNT haplotypes was not found in any of the isolates. The prevalence of pfcrt mutation between 2004 and 2006 was approximately 60% in Senegal and Kenya, as reported previously. In our study, we found a decrease in the prevalence of pfcrt gene resistance compared to previous level in Senegal and in Africa. However this level remains high with percentages of 48% observed in 2009.

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PREVALENCE OF MOLECULAR MARKERS OF SULPHADOXINE-PYRIMETHAMINE RESISTANCE IN AN AREA OF INTENSE, YEAR-ROUND MALARIA TRANSMISSION IN RURAL MALAWI

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Malaria infection in pregnancy is associated with severe maternal morbidity and increased perinatal mortality. Although sulphadoxine-pyrimethamine (SP) is no longer recommended as treatment for uncomplicated malaria due to resistance, SP is still recommended for intermittent preventive treatment in pregnancy (IPTp). Increasing resistance threatens the use of SP for IPTp. In 2010, we conducted cross sectional studies of the prevalence of molecular markers of resistance to SP among parasitemic patients presenting to the outpatient department (OPD) and delivery ward of Machinga District Hospital. In addition, pregnant women between 16 and 32 weeks of gestation with asymptomatic parasitemia were enrolled from antenatal clinic (ANC). Polymerase chain reaction was performed to examine molecular markers for SP resistance. Not all specimens could be amplified at all loci, therefore, percentages are given out of those that were amplified. We enrolled 196 OPD attendees and 291

pregnant women: 245 from ANC, and 46 at delivery. Primigravidae made up 44% of those from ANC and 59% of those at delivery. The overall prevalence of double (Gly-437/Glu-540 dhps), triple (Asn-108/Ile-51/Arg-59 dhfr) and quintuple mutants (double plus triple) was high (98%, 93%, and 92%, respectively), with no statistical difference among the groups. The prevalence of dhfr 164 was low (2%). The prevalence of dhps 613 was higher in OPD attendees than pregnant women (19% vs 5.4%, p-value=0.003). The prevalence of dhps 581 was high among pregnant women at delivery (37% vs 2.6% at ANC and 1.3% in OPD, p-value <0.0001). In this study characterizing molecular markers of *Plasmodium falciparum* resistance to SP in Malawi, the prevalence of the quintuple mutant was high, while the prevalence of dhfr 164 remained uniformly low. The prevalence of dhps 581 is significantly higher among pregnant women at delivery, suggesting that IPTp with SP during pregnancy is selecting for this mutation. Given the high levels of molecular resistance to SP, we need to develop new tools for preventing malaria in pregnancy.

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HIGH PREVALENCE OF PFCRT, PFDHPS AND PFDHFR DRUG RESISTANT HAPLOTYPES IN THE SOUTH BUT NOT IN THE NORTH OF CÔTE-D'IVOIRE

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The national malaria treatment policy changed twice in 2003 from Chloroquine to Amodiaquine, then in 2005 (adoption of ACT). However, the gap between the time of decision and the effective implementation of switch to ACT favored an abusive used of CQ and SP. The nationwide coverage of recommended ACTs is not quite effective due in part to disparities in the distribution of heath care infrastructures which are more concentrated in the south and mainly in Abidjan the economical capital of Cote-d'Ivoire. The level of resistance to CQ and SP in the country may greatly varies according to local usage of these two drugs. A prospective study was undertaken in Côte-d'Ivoire in 2008–2009 to assess by means of the pyrosequencing technology, the distribution of allelic frequencies of molecular markers associated with resistance to CQ and SP between two sites from the south and one site from the north, in 2008, after the changes of treatment policy for acute *Plasmodium falciparum* malaria in Côte-d'Ivoire. A total of 123, and 86 samples were collected from two southern sites, Ayamé and Anonkoua-kouté respectively, and 121 samples were collected from, Dabakala up north. Out of the samples collected from each of the three sites, 98 samples from the district of Ayamé were successfully amplified, 80 from Anonkoua-kouté, and 117 from Dabakala. As major finding this work points out that in 2008, the prevalence of the three key resistance-conferring haplotypes, the triple mutants Pfcrt IET and Pfdhfr IRN, and the simple mutant Pfdhps SGK, were higher in Ayamé and Anonkoua-kouté in the south while the sensitive haplotype Pfcrt MNK significantly predominated in Dabakala. The triple mutant IRN was rare at North. While ACTs are strongly recommended in the country to treat malaria, our work indicates a variable CQ and SP pressure nationwide. Mainly, these two drugs are still in used in the south where pressure is higher than up north. There could be a variable level of compliance to malaria treatment recommendations from the malaria control program.

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INVESTIGATING THE ROLE OF CANDIDATE MOLECULAR MARKERS OF LUMEFANTRINE AND AMODIAQUINE RESISTANCE IN CLINICAL OUTCOMES OF ARTEMISININ COMBINATION THERAPIES (ACT) OF *PLASMODIUM FALCIPARUM* MALARIA

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The initial reduction in peripheral *Plasmodium falciparum* parasitemia following artemisinin-based combination therapy (ACT) is driven predominantly by the potency and rapid action of the artemisinin component, but overall efficacy requires sustained therapeutic concentrations of the longer-acting partner drug. Candidate molecular markers associated with resistance in the pfcrt and pfmdr1 genes of *P. falciparum* have been reported to be involved in decreased sensitivity to amodiaquine and lumefantrine. However, the utility of these markers for predicting therapeutic responses to artesunate-amodiaquine and artemether-lumefantrine remains unclear. Correlation studies are confounded by the overall high PCR-corrected parasitological cure rates associated with these ACTs and by regional variation in immunity and parasite genetic background. Seventeen research groups have pooled their data on treatment outcomes and candidate resistance markers from efficacy studies conducted in different parts of the world. A total of 27 studies with 5,300 patients in 15 countries were included in the analysis. Our objectives were to investigate whether known polymorphisms in *P. falciparum* can predict treatment outcomes following artemether-lumefantrine and artesunate-amodiaquine therapy, and to determine whether resistance-associated genotypes are selected in recurrent infections. We have investigated associations between polymorphisms in pfcrt and pfmdr1 at the time of treatment and parasite clearance, recurrence, and PCR-determined recrudescence and reinfection. We have also assessed early and post-treatment selection of resistance-mediating genotypes. The results of these pooled analyses will clarify the roles of molecular markers for partner-drug resistance in monitoring ACT efficacy and will help to guide the selection of informative genetic markers in future studies.

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ASSOCIATION OF PFMDR1 AND PFCRT POLYMORPHISMS WITH SLOW CLEARANCE OF *PLASMODIUM FALCIPARUM* AFTER ARTEMISININ COMBINATION THERAPY IN WESTERN KENYA

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Artemisinin Combination Therapies (ACTs) are now considered the best treatment for *Plasmodium falciparum* malaria and have been widely deployed. A decline in the efficacy of artemisinin monotherapy in western Cambodia, characterized by slow parasite clearance, has recently been reported. The molecular mechanism of this reduced response to artemisinins has not been established. Artemisinins are thought to act within the parasite digestive vacuole and proteins found on the vacuole membrane may play a role in modulating drug sensitivity. Genes encoding two such proteins, pfcrt and pfmdr1, have been analyzed for sequence polymorphisms in samples collected during a clinical trial of artemether-lumefantrine (AL) and dihydroartemisinin-piperaquine (DHA-PIP) in Kenya in 2009. Genotypic analysis of the results showed significant associations between slow parasite clearance and CQ-sensitive haplotypes of pfmdr1 and pfcrt as measured by real-time quantitative PCR. The amino acid NFD haplotype at codons 86, 184, 1246 of pfmdr1 and CVMNK haplotype at codons 72-76 of pfcrt have also shown evidence of selection on day-3 after ACT treatment compared to day-0 prevalence. Previously published

data suggest that the selection of parasites carrying CQ-sensitive haplotypes of pfmdr1 and pfcrt could be attributed to the non-artemisinin partner drugs such as lumefantrine. This *in vivo* genotypic data in the present study supports the *in vitro* correlation between CQ-sensitive haplotypes of pfmdr1 and pfcrt and decreased sensitivity to artemisinins found in other studies. This study indicates for the first time that the CQ-sensitive haplotypes of pfmdr1 and pfcrt are associated with reduced response to artemisinins *in vivo*.

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EVALUATION OF COMMUNITY MALARIA WORKER PERFORMANCE IN WESTERN CAMBODIA: A QUANTITATIVE AND QUALITATIVE ASSESSMENT

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Village/ Mobile Malaria Workers (VMWs/MMWs) are a critical component in Cambodia's national strategy to reduce malaria morbidity and mortality. Since 2004, VMWs have been providing free malaria diagnosis and treatment using Rapid Diagnostic Tests and Artemisinin-based Combination Therapies in hard-to-reach villages (>5km from closest health facility). VMWs play a key role in control and prevention, diagnosis and treatment of malaria as well as in delivering behavioral change communication (BCC) interventions to this target population. To evaluate the implementation of these activities performed by VMW/MMWs, a quantitative and qualitative assessment was conducted in 5 provinces of western Cambodia in order to: (i) understand job satisfaction of VMWs and MMWs vis-a-vis their roles and responsibilities; (ii) assess their performance according to their job descriptions; and (iii) gain insights into the challenges faced in delivery of diagnosis, treatment and health education activities to their communities. A total of 196 VMWs/MMWs were surveyed in October 2011 using a combination of quantitative and qualitative methods. Triangulation of quantitative and qualitative data helped to gain a deeper understanding of the success factors of this intervention and the challenges faced in implementation. Overall, levels of VMW performance were in line with the expected performance (80%); however, some performance gaps were identified in the areas of knowledge of malaria symptoms, treatment regimens, and key messages. In particular, there were low levels of practice of the recommended direct observed therapies (DOTs) approach for malaria treatment (especially for the second and third doses), reportedly caused by stock-outs, distance and transportation. The national malaria program should aim to focus on improving knowledge of VMWs in order to address misconceptions and barriers to effective implementation of DOTs at community-levels. In addition to the findings, the tools developed, will potentially help the national program to come up with better indicators in the near future. Findings from this evaluation are being used to inform planning of future activities and interventions such as DOT in a context where artemisinin drug resistance is a significant public health issue.

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SELECTION OF *PLASMODIUM FALCIPARUM* STRAINS WITH REDUCED SENSITIVITY TO THE HIV PROTEASE INHIBITOR LOPINAVIR

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Some HIV protease inhibitors (PIs) are active against cultured malaria parasites at concentrations that are clinically relevant. Lopinavir acted against multiple laboratory strains of *Plasmodium falciparum* with an IC_{50} of 1-3μM, and against two freshly cloned strains from Tororo, Uganda with IC50 of 1.7μM. With standard dosing of lopinavir/ritonavir, lopinavir circulates at ~9-19 μM. Importantly, in an ongoing clinical trial, children treated for HIV infection with a lopinavir-ritonavir-based antiretroviral regimen experienced a 41% decrease in the incidence of malaria compared to children treated with a non-nucleoside reverse transcriptase-based regimen. Impacts of the PI on malaria were likely due principally to pharmacokinetic interactions between ritonavir and lumefantrine, but also the direct antimalarial activity of lopinavir. The antimalarial mechanism of action of HIV PIs is uncertain, although it is likely that they act against one or more of the ten plasmodial aspartic proteases known as plasmepsins. To help to characterize mechanisms of action and resistance, we selected malaria parasites with decreased sensitivity to lopinavir. We cultured the *P. falciparum* multidrug resistant reference strain W2 and the sensitive strain 3D7 with selective concentrations of lopinavir for fourteen months. Changes in sensitivity were selected only very slowly. The strains obtained after culture for 212 cycles under lopinavir pressure had IC_{50}s of ~10μM for both strains, corresponding to three times the IC_{50} of the parental strains. We are currently cloning parasites with reduced sensitivity to lopinavir and assessing the stability of the phenotype when drug pressure is removed. Differences between parasites with varied sensitivity will be assessed, including sequencing of plasmepsin genes. Our goal is to determine if alterations in lopinavir sensitivity selected in culture are due to specific genetic changes in plasmepsin genes or other portions of the *P. falciparum* genome. If alterations mediating changes in lopinavir sensitivity are identified, surveillance for these genetic determinants may help to guide optimal antiretroviral therapy in HIV-infected children at risk of malaria.

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MOLECULAR MARKERS OF ANTIMALARIAL DRUG RESISTANCE IN SOUTH-CENTRAL VIETNAM

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Malaria control programs introduced in Vietnam since 1990s have resulted in significant reduction in the number of malaria cases and deaths from the disease. Introduction of artemisinin derivatives and later artemisinin-based combination therapies (ACTs) for malaria treatment have contributed greatly to the overall success of this program. However, recent reports by WHO of reduced susceptibility of artesunate in Binh Phuoc Province in south Vietnam is of immense concern, which may hamper future efforts to control malaria in the country. Since 2006, we have conducted four drug efficacy trials (i.e. artemisinin-piperaquine, dihydroartemisinin-piperaquine and two formulations of artesunate-amodiaquine) in Phuoc Chien Commune, Ninh Thuan Province in south-central Vietnam for the treatment of uncomplicated *Plasmodium falciparum* malaria. The ACTs were found to be highly effective with PCR-corrected cure rates >98%. In the present study we have analysed the *in vitro* susceptibility of the field isolates collected in these trials to the commonly used antimalarials, as well as the polymorphism in

the major drug resistant markers: *pfcrt*, *pfdhfr-ts* and *pfmdr1*. Data on the prevalence of polymorphisms in these markers, as well as the drug susceptibility profiles of the clinical isolates will be discussed in the context of the ACT efficacy trials results. We also compared our findings at Ninh Thuan Province with published data from Binh Phuoc Province.

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CONTRASTING PATTERNS OF *PLASMODIUM VIVAX* MULTIDRUG RESISTANCE GENE 1 (PVMDR1) POLYMORPHISMS IN THAILAND AND CAMBODIA

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Plasmodium vivax remains a major source of malaria-related morbidity in Thailand and Cambodia. Data on drug resistance polymorphisms in *vivax* malaria populations from this region remains sparse. Studies have linked polymorphisms in the *P. vivax* multidrug resistance (pvmdr1) gene to chloroquine resistance and increase in pvmdr1 copy number to reduced susceptibility to mefloquine and other ACT partner drugs. In this study, we compared pvmdr1 resistance patterns between clinical isolates from northwestern Thailand and southern Cambodia collected between 2006 and 2009. Pvmdr1 copy number was quantified by a novel multiplex Taqman® real time PCR assay in 109 Cambodian and 49 Thai samples. Copy number was considered increased if the calculated value was greater than 1.7. Isolates were also sequenced to characterize the prevalence of two pvmdr1 mutant SNPs (Y976F and F1076L). In total, a greater proportion of Cambodian isolates harbored the 976F mutation correlated with chloroquine resistance (90% vs. 7.1%, p<0.001), while a greater proportion of Thai isolates displayed increased Pvmdr1 copy number (20% vs. 0.9%, p<0.001). Prevalence of double mutants was higher among Cambodian isolates than Thai isolates (95% vs. 7.7%, p<0.001). The 1076L single mutant was dominant among Thai samples; while both mutations occurred together in the vast majority of Cambodian samples. Our data highlight contrasting patterns of pvmdr1 polymorphisms in Thailand versus Cambodia. Selection for different Pvmdr1 haplotypes in these two areas has likely been shaped by different drug policies in the two countries. Further studies looking at the distribution of drug resistance alleles using microsatellites will help us gain a better understanding of the evolution of drug resistant *P.vivax* malaria.

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COMPARING CHANGES IN EFFICACY OF ARTESUNATE-MEFLOQUINE COMBINATIONS FOR THE TREATMENT OF UNCOMPLICATED *FALCIPARUM* MALARIA IN THAILAND DURING FIVE YEARS OF CLOSED DRUG MONITORING (2006-2010)

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The treatment of uncomplicated *Plasmodium falciparum* malaria in Thailand has been modified several times during the past 30 years to counter the rapid emergence and spread of drug resistance. This study was to compare the changes in efficacy of two-day and three-day combination of artesunate and mefloquine (ASM2 and ASM3). The study was conducted during 2006-2010 in 7 international bordered provinces to Burma, Cambodia and Malaysia. A total of 1,034 Uncomplicated *falciparum* malaria patients were enrolled in two phases, during 2006 and 2007, received ASM2 while those recruited during 2008-2010 received ASM3. All were followed for 42 days. This study found that the efficacy of artesunate-mefloquine combination was not only based on the drugs, but also the treatment regimen and variation of parasite genetics in different locations. Continuation of the monitoring of antimalarial drugs efficacies are necessary to cope up with the changing in efficacies.

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A RANDOMIZED TRIAL OF TEXT MESSAGE REMINDERS TO INCREASE ADHERENCE TO MALARIA TREATMENT

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Despite the massive international efforts made over the past decades, malaria continues to be one of the primary causes of under-5 mortality worldwide. Several recent studies document low adherence to artemisinin-based combination therapies (ACTs). Low adherence undermines the chances of patients fully recovering from acute malaria and increases the likelihood of the emergence of resistant strains of the parasite. We conducted a randomized controlled trial to investigate the impact of text message reminders on adherence to ACTs in Tamale, Ghana. One thousand one hundred forty participants were recruited from drug shops, licensed chemical sellers, public and private hospitals, and other ACT vendors when purchasing malaria medicine. Participants were randomized by automated system to the treatment group or the control group. Patients in the treatment group received six reminders, one for each dose of malaria treatment, sent out in 12 hour intervals. The primary outcome was adherence based on completion of treatment regimen. Adherence was assessed through observation of pill-packets and through self-reports at unannounced home follow-up visits timed to coincide with the completion of treatment. The follow-up rate was 99.6%. Receiving text message reminders increased the odds of adherence by 34% (95% CI [0.992-1.810], p-value 0.056) when a short message was used, and by 0% (95% CI [0.747-1.316], p-value 0.954) when a long message was used. Text message reminders appeared to work best when sent to the caretaker of child patients, with short messages increasing the odds of adherence by 125% (95% CI [1.299-3.890], p-value 0.004).

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THE INFLUENCE OF ENVIRONMENTAL RISK FACTORS AND INDIVIDUAL BEHAVIORS ON MALARIA OCCURRENCE IN LAHAD DATU DISTRICT OF SABAH, MALAYSIA: A CASE CONTROL STUDY

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Malaria is a parasitic disease and continue to be a major public health problem worldwide, it is estimated that 300 million people were infected by malaria with more than a million deaths throughout the world. In Malaysia, malaria incidence has decreased from 23.4 per 10 000 population in 1991 to 2.8 cases per 10 000 population in 2003. The incidence of malaria in the state of Sabah is the highest in Malaysia. In 2004, the incidence was 9.56 cases per 10 000 population which was the highest among the states. In the same year, Lahad Datu district recorded incidence of 24 cases per 10 000 population. Thus, this study was conducted to identify the factors which influence malaria infection in Lahad Datu district of Sabah. Malaria cases which were notified to district

health office from 1st January until 31st July 2008 which met with the inclusion criteria were included in the study. A total of 166 pairs of cases and controls which were matched by age, sex and mukim were analyzed. McNemar Odds Ratio (OR_M) calculated using McNemar Calculator for bivariate analysis while Conditional Logistic Regression was used for multivariate analysis. Data were coded and analyzed by using SPSS version 12.0. From the results, most of the malaria patients (95%) that included in the study were below 50 years old. 80.7% were male and only 19.3% were female. Based on OR_M it was found that socioeconomic status, risk perception, movement, preventive behaviours, house structure and design, and presence of potential mosquito-breeding place were risk factors for malaria infection. Multivariate analysis showed cases were more likely to have a high risk occupation (OR= 4.14, 95% CI= 1.04-16.57), low risk perception towards danger of malaria (OR= 6.01, 95% CI= 1.22-29.63), no residual spray done at home (OR=3.70, 95% CI=1.02-13.39) and have history of movement in the last two weeks (OR=5.25, 95% CI= 1.03-26.71). As a conclusion, some individual behaviours and preventive factors are strong risk factors for the occurrences of malaria cases in Lahad Datu district. With the identification of the potentially modifiable risk factors, a proper public health intervention can be implemented.

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ELEVATED INCIDENCE OF NON-*FALCIPARUM* MALARIA DURING THE RAINY SEASON 2011 IN NIGER

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Non-*falciparum* malarias are not benign disease states, but little attention is devoted to them in sub-Saharan Africa due to the prominence of *falciparum* malaria and its attendant complications. Most studies in sub-Saharan western Africa describe the frequency of non-*falciparum* malarias as 10% or less. Following implementation of a 2 step protocol utilizing both light microscopy (LM) and the addition of a malaria rapid diagnostic test (RDT) (Standard Diagnostics, Malaria Antigen, 05FK60) a retrospective review of laboratory records for 282 patients during a single four month rainy season was performed. Results indicated a higher than anticipated frequency of 24.6% positive tests for non-*falciparum* malaria species and 79.4% *falciparum*-positive tests. When LM alone was used for diagnosis, the rate of positive smears was 79%. When LM was combined with an RDT, the rate of positive tests decreased to 32%. With a population suffering from high prevalence of malnutrition and disadvantaged economic status, the Nigerien population is at significant risk from non-*falciparum* malarias. Potential reasons for an elevated incidence of non-*falciparum* malaria in this population are discussed.

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THE EVALUATION OF EASY ACCESS GROUPS AS A TOOL FOR MONITORING TEMPORAL CHANGES IN MALARIA TRANSMISSION AND COVERAGE OF CONTROL INTERVENTIONS IN MALAWI: THE EVALMAL STUDY

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Currently recommended tools for measuring progress of malaria control involve large, logistically and financially demanding population-based household surveys that provide national and provincial level estimates at intervals of 2 - 5 years. Since malaria transmission intensity and disease incidence can vary widely within a country though, programmatic decisions are often made at district level. With the change of focus from control of burden to reduction of transmission and the recent progress made, malaria trends in older (and asymptomatic) age groups become more important. Timely, valid, low-cost district and local level estimates of short- and medium-term control progress are urgently needed to support the move towards the control and elimination of malaria. Opportunistic sampling in planned or spontaneous aggregations of sub-groups of the population, the so-called Easy Access Groups (EAGs), offers the prospect of a less resource intensive method of deriving estimates of control progress. Children >4 months presenting at the EPI vaccination clinic at Chikwawa District Hospital, any accompanying older sibling(s) aged <15 years, and their parents/guardians were surveyed monthly since April 2011. A modified version of the RBM MERG MIS questionnaire will be administered to the parent/guardian. A finger blood sample was collected for a blood film, a malaria rapid diagnostic test, haemoglobin assessment and a filter-paper blood spot for serology. The estimates of burden of disease and uptake of control interventions were compared to that of a rolling Malaria Indicator Survey (rMIS) in the same population. The data presented is from the first year of the study (April 2011 - March 2012). The results will focus on the comparison of estimates derived from the EPI EAG and the rMIS. In conclusion, we determined if valid population level estimates of malaria intervention coverage and burden indicators and their short-term temporal trends can be obtained from opportunistic sampling in EAGs

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NON-RANDOM ASSOCIATIONS OF T-CELL EPITOPES IN *PLASMODIUM FALCIPARUM* CIRCUMSPOROZOITE PROTEIN, LILONGWE, MALAWI

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Cellular immunity to *Plasmodium falciparum* circumsporozoite protein (CS) is mediated by two immunodominant T cell epitopes (TH2 and TH3). Recent studies have found non-random associations between these epitopes in natural parasite populations, suggesting constraints on permissible TH2/TH3 combination haplotypes. Using sequence data from parasites from Lilongwe, Malawi (235 isolates) and The Gambia (44 isolates), we evaluated the extent of non-random association between the epitopes. In both populations, T-cell epitopes did not assort randomly. In fact, some combinations of TH2 and TH3 epitopes occurred more frequently than expected by random chance in both populations. The mechanism driving this deviation from random assortment is unclear; however it appears similar in parasite populations from East and West Africa. Potential explanations would include selection of certain combinations (by human immunity or within the mosquito host) or functional constraints on protein secondary/tertiary structure. Interestingly, among the Malawian isolates some of the most over represented combinations were highly similar to the T-cell epitopes contained in the RTS,S vaccine. In total, we found 118 (50.2%) isolates having at least a TH2 or TH3 epitope within one amino acid of the RTS,S vaccine type, while 230 (97.9%) have a TH2 or TH3 epitope within two amino acids of the RTS,S. Further characterization of this phenomenon is ongoing and is likely to be important in the design of next generation CS based vaccines.

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DIFFERING SIGNATURES OF SELECTION ON TWO *PLASMODIUM VIVAX* CANDIDATE VACCINE ANTIGENS

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To date, candidate vaccine antigens for *Plasmodium vivax* have primarily been selected based on their orthology to *P. falciparum* vaccine candidates. In several studies, *falciparum* antigens have shown genomic signatures of immune selection. However, few studies have evaluated if candidate *vivax* antigens show similar signatures of selection. We explored the genetic diversity of two *P. vivax* vaccine candidates, the circumsporozoite surface protein (pvcsp) and the merozoite surface protein 1 (pvmsp1), in a panmictic Cambodian *vivax* population, using scalable next-generation sequencing. We assessed these loci for evidence of selection and compared the results to similar analyses of other, geographically distinct *P. vivax* populations. In geographically diverse *P. vivax* populations, the 42-kd region of pvmsp1 consistently displayed a signature of strong balancing selection. Moreover, interspecies comparisons of orthologous antigens revealed that a subregion within the 42-kd block of pvmsp1 is under strong selection for non-synonymous nucleotide changes. In contrast to pvmsp1, the N-terminal and C-terminal conserved regions of pvcsp showed minimal evidence of balancing or directional selection. However, pvcsp sequences were highly heterogeneous, due to the central repeat region which appears to be under immune selection. As evidence, VK210 repeat arrays display a significantly higher proportion of non-synonymous nucleotide polymorphisms compared to VK247 arrays. In addition, repeat length polymorphisms appear to have occurred by a rapid and recent expansion as determined by mismatch distributions of the repeat arrays. These results demonstrate that immune selection on these antigens likely results in two different adaptive patterns, each increasing the genetic diversity of these candidate vaccine antigens in a different way. Similar to *falciparum* malaria, genomic approaches to detect alleles under immune selection may identify novel targets of immunity.

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THE EFFECT OF MALARIA AT DELIVERY ON FETAL ANEMIA AND THE ROLE OF INTERMITTENT PREVENTIVE TREATMENT DURING PREGNANCY IN MALAWI

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Fetal anemia is common in malarious areas and is a risk factor for infant morbidity and mortality. Malaria during pregnancy may decrease cord hemoglobin (Hb) and cause fetal anemia among newborns. Intermittent preventive treatment in pregnancy (IPTp) with sulfadoxine-pyrimethamine (SP) is protective against malaria, but has also been hypothesized to contribute to fetal anemia by affecting hematopoiesis. Peripheral, placental, and cord blood were examined for malaria parasitemia and hemoglobin concentration in a cross-section of 3,848 mothers and infants delivered at Queen Elizabeth Central Hospital in Blantyre, Malawi between 1997 and 2006. Unconditional linear and logistic regressions were performed with multiple imputation for missing covariates to assess the associations between malaria, IPTp with SP, and fetal anemia (cord Hb <12.5 g/dL). The overall prevalence of fetal anemia was 7.9% (n=304). Malaria parasitemia at delivery was associated with an decrease in cord Hb of 0.24 g/dL (95% confidence interval (CI): 0.05, 0.42), adjusting for SP use, gravidity, year, and season of delivery. The adjusted prevalence odds ratio (POR) for the effect of malaria on fetal anemia was 1.41 (95% CI: 1.05, 1.90). Primigravidae who did not take IPTp had infants at highest risk for fetal anemia (adjusted POR: 3.37; 95% CI: 1.68, 6.78), and density of parasitemia was correlated with a decrease in cord Hb of 0.33 g/dL (95% CI: 0.14, 0.53) and 0.35 g/dL (95% CI: 0.13, 0.57) per log increase in placental and peripheral parasitemia respectively. There was no significant association between SP use and cord Hb or fetal anemia (adjusted POR: 0.98; 95% CI: 0.69, 1.39). Malaria during pregnancy, but not IPTp, decreases cord Hb and is a risk factor for fetal anemia in Malawi. IPTp with SP may continue to be safe and effective in preventing malaria during pregnancy and fetal anemia despite development of SP resistance.

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MODELLING THE DEVELOPMENT OF ACQUIRED IMMUNITY TO *PLASMODIUM FALCIPARUM* MALARIA

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Development of immunity to malaria is a significant epidemiological process. It influences the transmission dynamics of the infection and disease, dictates how severe an infection will be and influences the evolutionary dynamics of the parasite due to host immune pressure. Despite the importance of immunity, it is unclear what mechanisms are involved in its development. The primary goal of this project is to describe the process of acquisition of immunity in a population with repeated exposure using a mathematical model of malaria transmission. A plethora of malaria control strategies have been implemented and others such as a malaria vaccine offer future prospects. The impact of these programmes on malaria transmission and subsequently the effect they have on the rate of development of immunity will also be evaluated in this study.

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GENOTYPING OF *PLASMODIUM FALCIPARUM* USING ANTIGENIC POLYMORPHIC MARKERS AND TO STUDY ANTI-MALARIAL DRUG RESISTANCE MARKERS IN MALARIA ENDEMIC AREAS OF BANGLADESH

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In the past many regions of Bangladesh were hyperendemic for malaria. Malaria control in the 1960s to 1970 eliminated malaria from the plains but the Chittagong Hill Tracts remained as a difficult to control reservoir. Northeastern Bangladesh with many tea plantations has malaria rates below 1% with approximately 10-20% *Plasmodium vivax.* The Chittagong Hill Tracts have areas with between 1 and 10% annual malaria rates predominately 90-95% *P. falciparum*. In southeastAsia multiplicity of infection for hypoendemic regions has been approximately 1.5. Few studies on the population dynamics of *P. falciparum* have been performed in Banlgadesh. Here we report polymorphic and drug resistant genotype analysis on 33 paired recrudescent infections after drug treatment in the period 2004-2008 in the Chittagong Hill Tracts which is just prior to countrywide provision of artemisinin combination therapy. Overall the multiplicity of infection for MSP-1 was 2.7 with a slightly smaller parasite diversity post-treatment. The 13 monoclonal infections by both GLURP and MSP-1 were evenly divided between pre- and post-treatment. The MSP-1 MAD block was most frequent in .66 of the samples. The prevalence of the K76T PfCRT chloroquine resistant allele was approximately 82% of the samples, while the resistant Pfmdr1 N86Y was present in 33% of the samples. Interestingly the post-treatment samples had a small but

significantly higher amount of the sensitive PfCRT alleles by RT-PCR. In conclusion, the parasite population retains a high population diversity despite hypoendemic transmission with retention but decrease in the chloroquine resistant allele and Pfmdr1 resistant alleles.

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POPULATION STRUCTURE AND SPATIAL DISTRIBUTION OF *PLASMODIUM FALCIPARUM* CIRCUMSPOROZOITE PROTEIN NANP REPEATS IN LILONGWE, MALAWI

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Humoral immunity to *Plasmodium falciparum* circumsporozoite protein (CS) is mediated by a central region of the protein containing a repetitive tetra-amino-acid repeat termed the "NANP repeat." Genetic analysis suggests that variants with different repeat lengths have spread recently in the population by a rapid mechanism such as slip-strand mispairing. It has been suggested that this is an adaptive mechanism of the parasite to evade immune recognition by the host. In some studies of RTS,S vaccine efficacy, levels of antibodies to this region of CS have been the most highly correlated marker of protective immunity. To date there have been no descriptions of the population structure of *P. falciparum* based upon differences in these repeats. Using filter paper blood spots from 100 participants in a study in Lilongwe, Malawi, we used capillary electrophoresis to determine the size of the NANP repeat region of parasite variants. Preliminary results confirm that infection by multiple genetically distinct variants of the parasite is common and that genetic diversity of *P. falciparum* infections is similar in adults and children. As each participant is geolocalized, this allows us to assess the spatial distribution of parasite variants, spatial variation in parasite diversity and the impact of environmental factors (such as proximity to water) in a multivariable spatial model of this diversity using our ArcGIS database. Isolation-by-distance among parasites has been suggested in *falciparum* malaria in the past. The impact of parasite genetic diversity on many critical issues for malaria control remains unclear. Investigating parasite population structure and diversity can help us better understand immunity, response to selective pressures and evolution of the parasite.

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GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY PREVALENCE IN THE GAMBIA

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Current malaria treatment guidelines recommend the use of primaquine as gametocytocidal treatment for *falciparum* malaria in settings targeting elimination. However, fears on the primaquine's potential hemolytic effect in individuals with glucose-6-phosphate dehydrogenase deficiency (G6PDd) have precluded its implementation, particularly in sub-Saharan African countries where the prevalence of G6PDd is either unknown or outdated. In this study, we present genotype and phenotype data in The Gambia and describe phenotype profiles for mutations with reported high prevalence in the Senegambia region. Filter paper blot spots from 3,100 healthy children aged 6-14 years collected during a school survey were analysed. Enzyme activity was determined quantitatively with a commercial test Kit (Atlas Medical®) and results adjusted for individual haemoglobin level. The frequencies for the A (A376G) and A- variant mutations; G202A, T968C and A542T were determined using Taqman® assays. The correlations between genotype and enzyme activity was also studied. Fifty-two percent of children were male and the mean haemoglobin was 12.4 (SD 1.3) g/dl. Preliminary analysis showed median activity of 6.5 (range 0-22) U/g/Hb in the analysed subset. The prevalence the A (A376G) and A- (A202G) mutations was 37.8% and 4.0%, respectively. The wide range of enzyme activity observed together with a low prevalence of the A- (A202G) genotype suggests that phenotype-based assessment may be needed before wide scale use of primaquine.

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RESPONDENT-DRIVEN SAMPLING ON THE THAILAND-CAMBODIA BORDER. I. CAN MALARIA CASES BE CONTAINED IN MOBILE MIGRANT WORKERS?

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Reliable information on mobility patterns of migrants is a crucial part of the strategy to contain the spread of artemisinin-resistant malaria parasites in Southeast Asia, and may also be helpful to efforts to address other public health problems for migrants and members of host communities. In order to limit the spread of malarial drug resistance, the malaria prevention and control programme will need to devise strategies to reach cross-border and mobile migrant populations. The Respondent-Driven Sampling (RDS) method was used to survey migrant workers from Cambodia and Myanmar, both registered and undocumented, in three Thai provinces on the Thailand-Cambodia border in close proximity to areas with documented artemisinin-resistant malaria parasites. 1,719 participants (828 Cambodian and 891 Myanmar migrants) were recruited. Subpopulations of migrant workers were analysed using the Thailand Ministry of Health classification based on length of residence in Thailand of greater than six months (long-term, or M1) or less than six months (short-term, or M2). Key information collected on the structured questionnaire included patterns of mobility and migration, demographic characteristics, treatment-seeking behaviours, and knowledge, perceptions, and practices about malaria. Workers from Cambodia came from provinces across Cambodia, and 22% of Cambodian M1 and 72% of Cambodian M2 migrants had been in Cambodia in the last three months. Less than 6% returned with a frequency of greater than once per month. Of migrants from Cambodia, 32% of M1 and 68% of M2 were planning to return, and named provinces across Cambodia as their likely next destinations. Most workers from Myanmar came from Mon state (86%), had never returned to Myanmar (85%), and only 4% stated plans to return. In conclusion, information on migratory patterns of migrants from Myanmar and Cambodia along the malaria endemic Thailand-Cambodian border within the artemisinin resistance containment zone will help target health interventions, including treatment follow-up and surveillance.

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MALARIA AND GRAVIDITY INTERACT TO MODIFY MATERNAL HAEMOGLOBIN CONCENTRATIONS DURING PREGNANCY

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Since the implementation of intermittent preventive treatment (IPTp) in sub-Saharan Africa, the effect of malaria-focused preventive measures on anaemia in relation to gravidity has been seldom investigated. We analysed data from 3 studies carried out in nearby areas in south Benin between 2005 and 2012. At inclusion (ANV1) women's age, area of residence, schooling, parity, gestational age, weight and height were recorded. Thick blood smears were performed on ANV1, second visit (ANV2) and at delivery. Women's serum ferritin and CRP concentrations

were also assessed. The impact of gravidity on maternal haemoglobin (Hb) was analysed using a logistic or linear regression depending on the outcome. The statistical significance was set to P < 0.05.
The study was approved by the Ethics Committee of the Faculty of Medicine of Cotonou in Benin. In total, data from 3591 pregnant women were analysed. Both univariate and multivariate analyses showed a constant association between Hb concentrations and gravidity in the 3 periods of Hb assessment (ANV1, ANV2 and delivery). Mean Hb concentration was significantly lower in primigravidae than in multigravidae at ANV1 (mean difference = -2.4 g / L, P < 0.001). Afterwards, it increased importantly in primigravidae only, with a tendency to reversal between primigravidae and multigravidae which was confirmed at delivery (mean difference = 2.8 g / L, < 0.001). The prevalence of malaria was halved between ANV1 and delivery in primigravidae while it decreased only by 38% among multigravidae, who were less prone to be infected (malaria prevalence at ANV1, 20% and 10% respectively). Iron deficiency was more common in multigravidae, and it decreased slightly in this group between ANV1 and delivery. In a context of IPTp, primigravidae were shown to improve progressively haemoglobin concentration throughout pregnancy. In multigravidae, the improvement was less perceptible as anaemia was mainly due to iron deficiency. There is a need to reinforce malaria prevention strategies in both groups

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ASSESSING THE ASSOCIATION BETWEEN MALARIA CHEMOPREVENTION AND THE NUTRITIONAL STATUS OF A COHORT OF YOUNG AFRICAN CHILDREN

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Malaria and malnutrition are common causes of morbidity and mortality in African infants. Data are limited as to whether antimalarial chemoprevention improves nutritional status. We compared the nutritional status of 393 infants living in Tororo, Uganda and randomized to 4 antimalarial chemoprevention arms at 6 months of age; no therapy, monthly sulfadoxine-pyrimethamine (SP), daily trimethoprim-sulfamethoxazole (TS) or monthly dihydroartemisinin-piperaquine (DP). Anthropomorphic measures were made monthly and the primary outcomes of interest were a drop of > 1 standard deviation (SD) in the height-for-age (HAZ) and weight-for-age (WAZ) z-scores from 6 to 18 months of age. Covariates of interest included breastfeeding status, maternal age, household wealth and chemoprevention arm. Associations between worsening nutritional status and covariates of interest were estimated using multivariate logistic regression. Mean baseline HAZ and WAZ scores were -0.98 and -0.42, respectively. From 6 to 18 months of age, 45% and 23% of infants had a drop of > 1 SD in their HAZ and WAZ scores, respectively. Continued breastfeeding at 18 months was protective against a > 1 SD drop in the HAZ (OR=0.53, p=0.04) and WAZ (OR=0.18, p<0.001) score. Compared to a maternal age of over 25 years, a maternal age of 18 years or younger was protective against a > 1 SD drop in the HAZ (OR=0.30, p=0.001) and WAZ (OR=0.36, p=0.03) score. There were no significant associations between household wealth or chemoprevention and worsening nutritional status and with the exception of a trend towards a lower odds of a > 1 SD drop in the WAZ score (OR=0.46, p=0.06) among infants randomized to monthly SP compared to those randomized to no therapy. In this cohort of infants living in a rural area of Uganda with high malaria transmission intensity, chemoprevention did not clearly improve nutritional status but sustained breastfeeding and younger maternal age were protective against worsening nutritional status from 6 to 18 months of age. Results will be updated through September 2012 when all infants have reached 24 months of age.

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MALARIA TREATMENT COST IN HEALTH SYSTEM: WHAT IS THE CHILDREN UNDER FIVE YEARS OLD MALARIA PROVIDER COST IN BURKINA FASO (WEST AFRICA)?

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Malaria is the major cause of morbidity in Burkina Faso especially among children under 5 years old. The cost related to the treatment of this disease in the country has not been well documented at the household and health system levels. Knowledge about the cost of treating malaria can affect the health care seeking behaviour of people and the use of different malaria prevention products. This paper estimates the health system cost due to simple or severe malaria with children under 5 years old in order to make available better understanding of the burden of malaria. Data have been collected from the following health facilities: the Nanoro religious district hospital, primary health facilities (5). We reviewed also Medical outpatient (243) and inpatient (122) records. We interviewed 46 Outpatient (OPD) and 10 inpatient (IPD) caregivers. Health system cost was estimated per component drug and lab test, personnel, and building. Malaria was ascertained not by parasitological tests but through fever at primary facilities. Lab test was used at district hospital. The survey was conducted from July to September 2010, during the high transmission season. Simple malaria unit cost for OPD at facility level was 1.9 USD for medicine, 0.2 USD for lab test, 0.3 and 0.4 USD for nurse at primary centre and district hospital, 0.3 and 1.0 USD for infrastructure at primary and district hospital. For severe malaria, IPD cost at district hospital was 4.5 USD for medicine, 7.2 USD for consumable, 18.7 for lab test, 2.0 for nurse, 6.2 for MD and 4.5 for building. The average cost of treating an episode of the disease including direct and indirect costs for household was 8.5 USD at OPD exit interview and 71.19 USD at IPD exit interview. For simple malaria, drug cost was the highest. Laboratory tests had the highest unit cost of severe malaria followed by consumables and personnel (MD) cost. Simple malaria cost without co-morbidity was 1.57 USD, with one co-morbidity 2.52 USD and with 2-3 co-morbidities 3.96 USD. Severe malaria cost without morbidity was 19.12 USD, with one co-morbidity 23.60 USD and with 2-3 co-morbidities 25.23 USD. In conclusion. malaria cost for health system is higher with co-morbidities. The introduction of prevention measures could reduce the cost of the treatment of malaria. In addition, the better implementation and monitoring of abolition of user fees policies could reduce the morbidity of malaria.

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MATERNAL ANEMIA IN PREGNANCY: ASSESSING THE IMPACT OF PREVENTIVE MEASURES IN A MALARIA ENDEMIC AREA

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Although widely implemented, the effectiveness of anaemia preventive measures (intermittent preventive treatment in pregnancy (IPTp), anti-helminthic and haematinics) on maternal anaemia at different time points of gestation in sub-Saharan Africa still need to be documented.
1005 pregnant women participating in a clinical trial of IPTp were followed from early pregnancy until delivery between 2010 and 2012 in southern Benin, where malaria transmission is perennial. On inclusion (ANV1), baseline characteristics of the women were recorded. At ANV1, the second antenatal visit (ANV2) and delivery, gestational age was assessed and anthropometric measurements were made. The first and second intakes of IPTp were given on ANV1 and ANV2 under supervision. A treatment dose of albendazole and haematinics were given at ANV1 to be taken home. At all time points, haemoglobin (Hb) levels, malaria and helminth infections were determined. Serum iron, folate, vitamin B12,

CRP concentrations were also measured. The effectiveness of preventive measures on the risk of anaemia and Hb concentrations was assessed at ANV2 and delivery by comparing the risk factors between ANV1 and after interventions (ANV2 and delivery). Multivariate linear and logistic regressions were used as appropriate. 63.8% of the women were anaemic at ANV1, 64.7% at ANV2 and 40.6% at delivery. The prevalence of malaria decreased from 15.1% at ANV1 to 4.0% at ANV2, and increased again at delivery to 9.6%, malaria infection being associated with a lower mean Hb at ANV1 and delivery. Helminth prevalence decreased from 11.1% at ANV1, to 7.2% at ANV2 and 2.4% at delivery. Iron deficiency stayed high throughout pregnancy (33.3% at ANV1, 36.3% at ANV2 and 30.7% at delivery). IPTp and anti-helminthic treatments were efficacious to clear parasitic infections and to improve haematologic status, whereas the effectiveness of daily iron and folate supplements to correct iron and folate deficiencies and to decrease anaemia was less marked.

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A PILOT SCHOOL SURVEY TO ESTIMATE THE MALARIA BURDEN IN A SETTING WITH DECLINING ENDEMICITY

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In a setting with declining malaria endemicity such as The Gambia, identification of malaria infected individuals is increasingly harder. School surveys may represent an easy and inexpensive method to identify foci of malaria transmission. The aim of this study was to evaluate the use of school surveys to estimate the malaria burden and identify foci of transmission, in the population. We carried out a school survey in the Upper River Region, The Gambia, in May-June 2011, before the start of the malaria transmission season. Thirty two primary schools were selected with probability proportional to size and in each of them 100 pupils were randomly enrolled. Data on socio-demographic variables and was collected through a questionnaire. A blood sample was collected for 1) detection of antimalarial antibodies against merozoite surface protein (MSP) 1, MSP2, and apical membrane antigen-1 (AMA-1) by ELISA, 2) microscopy (asexual forms and gametocytes), 3) PCR detection of malaria infection, and 4) haemoglobin by Hemocue. Three thousand two hundred seventy seven children (48% girls) were included in the survey. The mean age was 10 years (range: 4-21). Bed net use was 73%. About 17% had a history of fever in the past 48 hours while 3% had fever (axillary temperature ≥37.5ºC) at the time of the survey; none was positive by rapid diagnostic test. About 11% of the children had anaemia (haemoglobin<11g/dL). The parasite prevalence was 10% (309/2681) for *Plasmodium* genus, and 9% (277/2871) for *P. falciparum* species. There was evidence of heterogeneity in the parasite prevalence across schools. In addition there was heterogeneity in age, reported use of bed nets, and anaemia across the schools. School survey data can be used to determine the malaria burden and identifying foci of malaria transmission in regions of declining endemicity. These foci will be sites for other studies to determine the cause of heterogeneity and interventions that can contribute to malaria elimination efforts.

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THE MALARIA HOUSEHOLD COST OF CHILDREN UNDER FIVE YEARS OLD IN BURKINA FASO (WEST AFRICA)

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An estimated 700,000-2.7 million persons die of malaria each year, 75% of them African children . This disease is the major cause of health facilities use. This paper estimates the direct and indirect cost of simple and severe malaria for under 5 years children for household in order to provide a better understanding of the burden of malaria to household. Data have been collected from 506 households of 24 villages of Nanoro's demographic surveillance site in Burkina Faso. A random sampling of household was done. We included household with children under five years old and we exclude children enrolled on the malaria vaccine trial. The obtained informations has beene used to estimate the direct and indirect costs of malaria treatment. The direct cost of malaria treatment includs all cash expenditures on seeking malaria care by patients and their caretakers. The indirect costs included all cash expenditures on transportation and non medical supplies. Households were interviewed about their expenditure on malaria treatment for children under 5 years old. Simple random sampling was used to select villages and households with at least one child under 5 years old. Malaria was ascertained not by parasitological tests but through fever using a recall period of one month. The survey was conducted during the high transmission season in 2010. Durinh household survey, the average cost of treating an episode of the disease including direct and indirect costs for household were 7.83USD. During exit interview this cost were evaluated 8.5 USD at OPD and 71.19 USD respectively at Outpatient OPD and Inpatient IPD. The average total cost for rich households was higher than the poorest one. The productivity cost was 6.01 USD at household survey, 5.72 USD at OPD and 37.71 USD at IPD exit interview. In conclusion, there is an equity access of malaria care for children under 5 years old.. Productivity cost are the most important for household.Indirect cost reduction will contribute to individuals and family well being

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DRUG THERAPY OF SUSPECTED MALARIA CASES BEFORE THEIR ADMISSION IN A DISTRICT HOSPITAL IN BURKINA FASO DURING THE DRY SEASON

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Misuse of antimalarials drugs has led to the emergence of resistant *Plasmodium falciparum* strains. Malaria treatment protocols were reviewed at the beginning of 2000 in African countries and artemisinine based combination therapy (ACT) was introduced. To describe the treatment itinerary of suspected cases of malaria before their admission to the district hospital of Do, seven years after the introduction of ACT in Burkina Faso. From December 2010 to May 2011, we conducted a cross-sectional survey of suspected malaria cases admitted at the district Hospital during the dry season (malaria low incidence season). We included all patients aged 6 months or above, recorded as suspected malaria according to the criteria of national malaria control program, excluding those with chronic defects. 476 suspected cases, out of which 422 (88.7%) uncomplicated and 54 (11.3%) complicated, were recruited, representing 7.9% of the admissions. The number of cases decreased from December (207 cases) to May (14 cases) with a monthly average of 79 cases. The average age was 14.4 years, ranging from 6 months to 76 years. Cases under 5 years were 168 (35.3%). Treatment itineraries were mainly: initial consultation in a health facility of first resort (public or private clinic), 20 cases (4.2%); direct consultation to the district hospital, 104 cases (21.8%) ; initial consultation with a traditional healer, 3 cases (0.6%); initial self-medication, 346 cases (72.7%); out of the latter, 331 cases (95.6%) then consulted directly at the district hospital. The practice of self-medication did not differ between those aged less than 5 years and those above 5 years and over (OR = 0.6, 95% IC = 0.4 - 1.0), or by gender (OR = 1.2, 95% IC = 0.8 - 1.9). Self-medication drugs involved were mainly antipyretics (n = 327) and antimalarials (n = 58). Out of the latter, ACT was used in 39.6% of cases, quinine in 19.0% and non-recommended antimalarials, such as sulfadoxine-pyrimethamine, amodiaquine and chloroquine, in 41.4%. A total of 112 cases (23.5%) had positive thick blood smear, including 18

cases (16.1%) who had taken an antimalarial. During the dry season, the treatment itinerary of suspected malaria cases is marked by a short circuit at health care level and use of non recommended antimalarials by self-medication. Complementary analysis of the itinerary during the epidemic season may help to define more appropriate strategies to sensitize the population.

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REGIME SHIFTS, HETEROGENEOUS TRENDS AND INDIAN OCEAN DIPOLE INDUCED SYNCHRONY IN MALARIA TIME SERIES FROM KENYAN HIGHLANDS

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Large malaria epidemics in the East African highlands during the mid and late 1990s kindled a stream of research on the role that global warming might have on malaria transmission. Most of the inferences using temporal information have been derived from a malaria incidence time series from Kericho. Here we examined whether observed patterns in that time series were common across other localities in the lake Victoria basin of Western Kenya. We found that temporal trends were decreasing yet heterogeneous. Time series from localities above 1600 m showed regime shifts that coincided with the 1998 Indian Ocean Dipole, IOD. We found all the time series to more closely follow the interannual patterns of Variability of the IOD than El Niño Southern Oscillation, and we found the time series had a synchronous pattern that resembled a Moran effect, i.e., their patterns of concerted fluctuation were higher than the observed environmental correlation. The heterogeneity in malaria trends probably reflects the multitude of factors that can drive trends of malaria transmission and highlights the need for both spatially and temporally fine-grained data to make sound inferences about the impacts of climate change on secular changes in malaria transmission. Nevertheless, synchronous malaria epidemics call for the integration of knowledge on the forcing of malaria transmission by environmental variability to develop robust malaria control and elimination programs.

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ASYMPTOMATIC *PLASMODIUM* SPP. INFECTION AND COGNITION AMONG PRIMARY SCHOOLCHILDREN AGED 6-14 YEARS IN A HIGH MALARIA TRANSMISSION SETTING IN UGANDA

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In areas of high malaria transmission, asymptomatic *Plasmodium* infection is commonplace among school children, yet little is known about its impact on children's cognitive function. We investigated the association between asymptomatic *Plasmodium* infection and measures of sustained attention and abstract reasoning among primary school children in Tororo district, Uganda, a high malaria transmission area. In randomized placebo controlled trial assessing the impact of intermittent preventive treatment for malaria on morbidity and cognitive function, 740 children were enrolled. A detailed history and physical examination was conducted. Stool samples were examined for helminth infections and blood smears for malaria parasites. Two tests of cognition were administered to children: Raven's matrices for abstract reasoning and code transmission tests for sustained attention. Differences in mean test scores were analysed using t-tests and multivariable linear regression models. Of the 740 children at baseline, the mean (SD) age was 9.9 (4.3) years and 53.3% were females. The prevalence of *Plasmodium* spp. infection was 30.1%, with the majority of infections due to *P. falciparum*. Ninety percent of children reported coming from a household with at least one mosquito net but net use the previous night was only 36%. Children with *Plasmodium* infection had significantly lower mean scores in tests of abstract reasoning (37.6 versus 42.7 $p<0.0001$) and sustained attention (42.2 versus 49.5 $p<0.0001$) compared to uninfected children. Other factors significantly associated with poor scores in the cognitive function tests included sex, age and weight. In conclusion, despite high household coverage of mosquito nets, net use is unacceptably low among schoolchildren and *Plasmodium* infection remains highly prevalent and is strongly associated with poor cognition in schoolchildren. Such results underline the need for targeted approaches to malaria prevention and control in schoolchildren.

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PREVALENCE AND CORRELATES OF MALARIA PARASITEMIA IN PEOPLE LIVING WITH HIV/AIDS ATTENDING THE LAQUINTINIE HOSPITAL IN DOUALA, CAMEROON

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A substantial number of people living with HIV/AIDS (PLWHA) inhabit areas of high malaria transmission. To provide data to improve the prevention and care of malaria in such patients, we assessed the prevalence and socio-demographic and clinical correlates of malaria parasitaemia in PLWHA. Between April-June 2010, a cross-sectional study of adult PLWHA attending the Douala Laquintinie Hospital was conducted. After obtaining consent, socio-demographic and clinical data were obtained via a standardized questionnaire. Malaria parasitaemia was determined by blood smear microscopy. To determine correlates, means were compared using t-tests while proportions were compared using chi-square tests. The 238 PLWHA enrolled had a mean age of 40.8(±10.5) years. Most (67.6%) were females, 48.3% were on antiretroviral therapy and 41.4% had CD4+ counts<200 cells/ml. The proportion of participants who reported using bed nets and insecticides were 36.1% and 37.4% respectively. Overall, the malaria prevalence was 24.8%. Malaria prevalence was not significantly lower in patients using bed nets (23.3%), using insecticides (23.6%), nor in those on antiretroviral therapy (24.4%). Although malaria prevalence was higher in patients with CD4+ counts <200 cells/ml (30.6%) compared to those with CD4+ counts ≥200 cells/ml (20.1%) this did not achieve statistical significance (P=0.07). Malaria parasitaemia was prevalent in this population of PLWHA. Very few patients reported using preventive methods and even then, the self-reported measures taken to prevent malaria did not seem to be effective. Because of the potential for worse HIV outcomes in the presence of malaria (even when asymptomatic), malaria prevention and treatment (if indicated) needs to be reinforced.

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MALARIA GAMETOCYTE PREVALENCE IN NORTHERN KWAZULU-NATAL, SOUTH AFRICA

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South Africa has embarked on the ambitious goal of halting malaria transmission within its borders by 2018. If this goal is to be attained, all malaria cases must be detected and effectively treated. It has been suggested that primaquine become standard first line treatment together with artemether-lumfantrine to prevent onwards transmission. This suggestion has raised some health concerns as primaquine has been associated with heamolysis in glucose-6-phosphate dehydrogenase (G6PD) deficient individuals. To address this issue a pilot study to assess gametocyte carriage in northern KwaZulu-Natal was conducted during 2011/2012 malaria season. The Umkhanyakude Municipality of KwaZulu-Natal, South Africa was selected for the study due to its relative high malaria prevalence. Filter paper finger prick blood spots were collected during a community based survey. Parasite mRNA extracted from the

filter paper samples was subjected to a gametocyte specific reverse transcriptase PCR. Results from this study will be used to inform a policy on primaquine usage in KwaZulu-Natal.

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AN EVALUATION OF CHART ABSTRACTION TO ASSESS THE QUALITY OF CASE MANAGEMENT FOR INPATIENTS WITH SEVERE MALARIA - BENIN, 2010

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Ensuring high quality care for inpatients with malaria is critical. Chart abstraction offers a potentially efficient method to assess quality of care. However, as the availability and quality of inpatient charts in Benin was unknown, we conducted a study of the feasibility of sampling and abstracting charts, the validity of abstracted data, and the extent of missing data. Chart abstraction was conducted in July 2010 from a probability sample of inpatients (any age) in five Beninese hospitals (method 1). We also compared abstraction to interviews with health workers (HWs) and their patients admitted 12-48 hours earlier (N=11) (method 2), and interviews with HWs regarding patients they discharged within 72 hours (N=10) (method 3). Analysis of all methods focused on 11 signs of suspected malaria and severe disease, test results, and treatment. For method 1, we sampled 4% (60/1383) of inpatients admitted in June 2010. Of 60 patients sampled, 45 (75%) charts were retrieved and abstracted; 43 suspected malaria cases were identified. Of 473 signs, 179 (37%) were documented in charts. In 74% (32/43) of charts, at least one sign was present to identify severe disease. Antimalarial treatment was documented in 81% of charts of patients with suspected malaria (35/43). Interviews of HWs and admitted patients (method 2) showed that 96% (45/47) of documented signs were valid. HW interviews regarding discharged patients (method 3) showed that 35% (19/55) of non-documented signs were not assessed by HWs. Malaria test results were documented in 65% of charts (41/63) (methods 1, 2, 3). Abstraction from inpatient charts was feasible, and documented data were valid. Despite poor documentation, data were sufficient to identify severe illness for three-quarters of patients. Charts contained moderate levels of testing and high levels of treatment information. This study was limited by small sample size and possible recall bias. We recommend chart abstraction for an inpatient survey and introducing a standard admission form as an intervention to improve documentation and quality of care.

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GENETIC DIVERSITY OF *PLASMODIUM FALCIPARUM* IN THE THREE MAJOR ECOLOGICAL ZONES OF GHANA

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Parasite genetic diversity information is useful for malaria control activities such as drug and vaccine failure rates. Parasite diversity varies with transmission intensity in varying geographical settings. Our objective was to describe the level of *Plasmodium falciparum* genetic diversity in the three ecological zones Ghana. A random selection of 379 baseline (Day 0) filter paper blood blots from children under five years, recruited between 2005-2008 during the monitoring of the efficacy of antimalarial drugs for the treatment of uncomplicated malaria in Ghana were genotyped. DNA was extracted; allele frequency and genetic diversity were investigated by nested PCR of the block three of the *MSP2*. The samples were drawn from towns in the three major ecological settings of Ghana: Navrongo (Sudan Savannah), Begoro and Bekwai (forest zone) and Cape Coast (Coastal Savannah). Differences in clonal diversity were observed that can be explained by geographical location. In general FC27 was the dominant allele in the Ghanaian parasite isolates in comparison with the 3D7 (Wilcoxcon sign rank z, 2.953: p=0.0031). There were significant differences in clonal diversity in the different ecological zones when the carriage of a single clone was compared with carrying two clones (Wilcoxcon sign rank z, 2.062:p=0,039), single clone vs. three clones (Wilcoxcon sign rank z, 2.717 p=0.007) and two clones vs. three clones (Wilcoxcon sign rank z, 2.953: p=0.003). This clonal diversity can be further explained by the differences in ecological zones, with the Sudan Savannah showing less diversity than the forest zone and the coastal savanna zone. Also, the forest zone showed more clonal diversity than the coastal Savanna and the two forest based towns did not show significant differences in clonal diversity. Greater genetic diversity was observed in the FC27 alleles than the 3D7 alleles which may translate into more diversity in the different ecological zones and population differentiations of the MSP2 alleles probably due to differences in ecological zones resulting in varying transmission intensity and out-crossing of parasite isolates during sexual reproduction.

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ADOLESCENT PREGNANCY AND THE RISK OF *PLASMODIUM FALCIPARUM* MALARIA AND ANEMIA - A PILOT STUDY FROM SEKONDI-TAKORADI METROPOLIS, GHANA

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The problem of malaria in adolescence has been surpassed by the immense burden of malaria in children, most especially less than 5. A substantial amount of work done on malaria in pregnancy in endemic regions has not properly considered the adolescence. The present study therefore aimed at evaluating the prevalence of *Plasmodium falciparum* and anaemia infection in adolescent pregnant girls in the Sekondi-Takoradi metropolis, Ghana. The study was carried out at four hospitals in the Sekondi-Takoradi metropolis of the western region of Ghana from January 2010 to October 2010. Structured questionnaires were administered to the consenting pregnant women during their antenatal care visits. Information on education, age, gravidae, occupation and socio-demographic characteristics were recorded. Venous bloods were screened for malaria using RAPID response antibody kit and Geimsa staining while haemoglobin estimations were done by cyanmethemoglobin method. The results revealed that adolescent pregnant girls were more likely to have malaria infection than the adult pregnant women (34.6% verse 21.3%, adjusted OR 1.65, 95% CI, 1.03-2.65, P = 0.039). In addition, adolescent pregnant girls had higher odds of anaemia than their adult pregnant women equivalent (43.9% versus 33.2%; adjusted OR 1.63, 95% CI, 1.01-2.62, P = 0.046). Taken together, these data suggest that adolescent pregnant girls were more likely to have malaria and anaemia compared to their adult pregnant counterpart. Results from this study shows that proactive adolescent friendly policies and control programmes for malaria and anaemia are needed in this region in order to protect this vulnerable group of pregnant women.

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INTERMITTENT PREVENTIVE TREATMENT OF MALARIA IN PREGNANCY (IPTP): EMPHASIS ON ADEQUATE DOSAGE AND TRIMESTER UPTAKE

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Intermittent preventive treatment in pregnancy (IPTp) with sulphadoxine-pyrimethamine (SP) has been adopted as policy by all countries in sub-Saharan Africa. However, studies on the post-implementation effectiveness and coverage of the therapy are being evaluated upon possible resistance that has been reported. This study assessed the effectiveness and uptake of the therapy by pregnant women attending antenatal care with some emphasis on trimesters and supposed doses taken. This cross-sectional study compared malaria and anaemia prevalence among 754 pregnant women using IPTp-SP with non-IPTp-SP users. The results showed that 57.8% (436/754) used IPTp-SP while 42.2% (318/754) did not. In general, 81.4% of the IPTp-SP users were malaria negative while 18.6% were malaria positive and those who received ≥2 doses had significantly reduced prevalence of malaria. Furthermore, of those that received IPTp-SP, 20.4% were in their 3rd trimester while 71.3% were in their 2nd trimester. However, only 3% of the pregnant women completed 3 doses while 30% completed the full ≥2 doses of IPTp-SP. In multivariate analysis, malaria infection in 3rd trimester was associated with increased odds of anaemia (adjusted OR 6.81, 95% CI, 1.19-38.94). IPTp-SP usage among pregnant women reduces malaria and its efficacy must be strengthened by proper dosage completion.

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DEVELOPING A MALARIA ENDEMICITY MAP FOR ETHIOPIA USING SEROLOGICAL INDICATORS OF PRIOR EXPOSURE TO *PLASMODIUM FALCIPARUM* AND *P. VIVAX*

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Ethiopia has a diverse ecology and geography that results in spatial and temporal variation in malaria transmission. Using evidence-based strategies to allocate the most appropriate interventions to different populations and transmission settings is crucial to sustaining reductions in malaria burden in Ethiopia, minimising epidemic risk, and eventually attaining elimination. Defining endemicity based on the detection of infection through light microscopy or antigen-detecting rapid diagnostic tests to define endemicity has limitations, due to highly seasonal transmission in many areas and presence of low density infections. Detection of *Plasmodium falciparum* and *P. vivax* antibodies was used to examine previous exposure to infection, a proxy to assess local transmission over a period of years. 197 cross-sectional school-based surveys among children aged five to 18 years were conducted in Oromia Regional State during the main transmission season. Key indicators were detection of parasites by light microscopy, and enzyme-linked immunosorbent assay (ELISA) to detect IgG against four antigens: *P. falciparum* glutamate rich protein (PfGLURP), *P. falciparum* merezoite surface protein (PfMSP), *P. vivax* merezoite surface protein (PvMSP) and *P. vivax* apical membrane antigen (PvAMA). Few schools (30/197) were found to have any *Plasmodium* infections by microscopy (prevalence range 0-15%), but a wide variation (range 0-56%) in school sero-prevalence was identified. Among 30 schools with 0% prevalence by microscopy also examined by ELISA, sero-prevalence range was 0-24%, demonstrating the ability of serologic markers to identify heterogeneity in recent transmission intensity at sites with few or no current *Plasmodium* infections among sampled individuals. The distributions of *P. falciparum* and *P. vivax* seropositivity across Oromia are described, together with development of geostatistical Bayesian models linking school seropositivity with meteorological and remotely-sensed environmental correlates to create a predictive endemicity map.

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DEVELOPING A SCHOOL-BASED MALARIA EPIDEMIC DETECTION SYSTEM IN ETHIOPIA

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Epidemics of malaria occur periodically in Ethiopia, often resulting in substantial morbidity and mortality. Effective surveillance and response systems are required to allow early identification of epidemics and minimise the impact on health of the population. The current strategy for epidemic detection in Ethiopia relies upon charting weekly malaria cases against a 'normal' calculated using the third quartile method from the previous five years of data. However, there are limitations to the quality and frequency of reporting and analysis of these data. The current study explores the use of simple, easy to measure indicators of malaria morbidity and infection at school level, including school absenteeism, with a view to identifying an indicator which can be used for an epidemic early warning system. It is expected that this approach will allow the community to notice unusual increases in malaria morbidity, prompting an in-depth investigation by district health officials. In locations with limited accessibility to district towns, this may overcome some of the challenges of the current health facility based epidemic detection system. A series of eight repeated cross-sectional school surveys were conducted at six sites in southern Ethiopia over the short malaria transmission season (April to June 2012), randomly selecting 110 children for each survey. Indicators collected were multi-species rapid diagnostic test result, microscopy examination of blood film, reported fever on survey day and in prior two weeks and measured axillary temperature. Participants were asked their normal frequency of school attendance and number of absences from school in the previous two weeks. School records of pupil absenteeism were collected on completion of the study, as well as routine data on clinical and confirmed malaria from health facilities serving the study population. Survey indicators and school absenteeism rates were compared to health facility data, in order to identify indicators which correlate with and offer a lead time over the standard malaria epidemic detection system.

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A NOVEL METHOD TO ASSESS THE SAFETY OF SULFADOXINE-PYRIMETHAMINE FOR INTERMITTENT PREVENTIVE TREATMENT IN INFANTS USING ROUTINE HEALTH FACILITY DATA FROM SOUTHERN TANZANIA

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Intermittent preventive treatment with sulfadoxine-pyrimethamine is recommended for malaria prevention in infants (IPTi-SP) in areas of moderate to high malaria transmission and where parasite resistance to SP is not high. Serious adverse events, including Stevens-Johnson syndrome (SJS), have been reported following SP exposure, but few infant-specific data exist. Within the context of a cluster randomized controlled trial of IPTi-SP in southern Tanzania, we captured routine health facility data on infant outpatient attendance from all health centres. Data included diagnosis, allowing classification of attendance for a non-scabies skin condition. We investigated the association of IPTi-SP with attendance for skin conditions using a number of methods, including the self-controlled case series method. This novel methodology allowed estimation of the relative incidence of attendance among infants presenting with a skin condition who had received SP for IPT-SP or for malaria treatment. Among these infants, the rates of attendance were compared between 'unexposed' and 'exposed' periods of time, using infants as their own comparison- thus adjusting for all measured and unmeasured time-fixed confounders. Based on previous studies and the half life of SP active ingredients, we defined an 'exposed' period as the 42 days following an SP dose, and compared whether rates during this period differed to those during an infant's 'unexposed' time. Data were available for 9880 infants over 12 months, with >8000 doses of SP received. No diagnoses of SJS were recorded. The incidence of attendance with a skin condition was 0.062/year among all infants, and 0.071/year among the 3983 infants who had received >=1 dose of SP for IPT-SP or malaria treatment. In total 239 infants attended for a skin condition and received at least one dose of SP, and these were included in the self-controlled cases series analysis. In comparison to the rate during 'unexposed' periods, the age-adjusted rate of attendance among these 239 infants during the 42 days after exposure was almost 50% lower suggesting no detectable increased rate of attendance for skin condition during the six weeks following an SP dose. These results provide reassurance about the safety of SP in infants from this setting, and provide a worked example of how the self-controlled case series method may be used to assess safety of interventions in developing countries using routine health facility data

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A PROSPECTIVE ANALYSIS OF *PLASMODIUM FALCIPARUM* INFECTION RISK FROM INFANCY TO ADULTHOOD IN AN AREA OF INTENSE, SEASONAL TRANSMISSION IN MALI

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Time to first malaria episode is often used as a measure of clinical immunity to *Plasmodium falciparum*. In contrast, the risk of *P. falciparum* infection irrespective of clinical disease can be measured by the time required to detect parasites in the blood by sensitive methods such as polymerase chain reaction (PCR). Pre-erythrocytic malaria vaccines aim to elicit a protective immune response against the sporozoite or liver stage of *P. falciparum* to prevent infection, but little is known about naturally acquired pre-erythrocytic immunity in endemic areas. Prospective studies that have assessed the risk of *P. falciparum* infection in endemic settings have failed to show correlations between antibodies to pre-erythrocytic antigens and infection, and evidence for age-dependent acquisition of protective immunity to infection is limited. To evaluate for evidence of naturally acquired pre-erythrocytic immunity, we conducted a prospective analysis of *P. falciparum* infection during a single malaria season from May to December 2011 in Kalifabougou, Mali. Of the 695 subjects enrolled in this study, 372 were uninfected by PCR at enrollment before the malaria season. Of these, we determined time to *P. falciparum* PCR positivity using dried blood spots collected at two-week intervals over the entire study period. We found no evidence that pre-erythrocytic immunity is acquired with increasing age/exposure. Unexpectedly, younger children (<4 years old) showed decreased risk of infection compared to older children (4-17 years old) when adjusted for sex, hematocrit, *Schistosoma* co-infection, sickle cell trait, and spleen size (hazard ratio 0.44; 95% CI, 0.32-0.60; p<0.0001). Based on these data, antibody responses to *Anopheles gambiae* salivary gland extract, pre-erythrocytic antigens, and blood-stage antigens are being measured before and after the malaria season in an attempt to determine if the observed decreased risk in younger children is due to differences in vector/parasite exposure versus other factors.

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MALARIA IN THE KINGDOM OF SAUDI ARABIA - IS ELIMINATION A REALISTIC GOAL?

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In 1998, after two exceptional years of heavy rain, the Kingdom of Saudi Arabia (KSA) suffered its worst epidemic of malaria, with total cases reaching 40,796. Almost 90% of these were locally acquired, and incidence reached 11/1000 in the main malarious areas of Asser and Jazan, in Southern KSA. Since then, KSA has scaled up vector control with IRS, ITNs and larviciding and improved on case management. Today's data tells a very different story: the number of autochthonous cases since 2008 has been less than 100 per year, just 4% of total cases and an incidence rate of <0.05/1000, far lower than the rate of 5/1000 that WHO

recommends a country should achieve before considering elimination. On this basis alone, the goal of malaria elimination is considered realistic. Moreover, transmission is now limited to the southern regions of Asser and Jazan, bordering the Republic of Yemen, where *Plasmodium falciparum* is the main cause of malaria and *Anopheles arabiensis* the main vector. However, moving from a control to an elimination programme requires a considerable effort to scale up monitoring and evaluation efforts. KSA is adopting state of the art tools to support M&E and decision-making, developed by the IVCC with financial support from the Bill and Melinda Gates Foundation. Using annual data records, we show how trends in malaria in KSA have changed over time due to the impact of vector control as well as climate. Case data for malaria areas are utilised to generate focal risk maps of disease and to determine outbreak alert thresholds. The prospects for malaria elimination in KSA and the relevance of the model being applied will be discussed objectively in relation to other countries pursuing or considering malaria elimination, both in the region and globally.

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LANDSCAPE GENETICS OF *FALCIPARUM* MALARIA IN GEOGRAPHICALLY DISPERSED CONGOLESE SITES AND IN UN PEACEKEEPING SOLDIERS RETURNING TO GUATEMALA

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Malaria is dispersed from place to place by movements of either mosquitoes or humans. A better understanding of the patterns of dispersion will aid in preventing its geographical spread. Using landscape genetics, which combines population genetics and spatial epidemiology, we studied the population and spatial structure of *Plasmodium falciparum* in the Democratic Republic of the Congo (DRC). The population structure of *P. falciparum* parasites was characterized among 117 isolates from the DRC, 40 isolates from Guatemala and 12 from Guatemalan United Nations peacekeeping soldiers who visited DRC in 2010 and were found to have *P. falciparum* parasitemia by either slide microscopy or polymerase chain reaction (PCR) analysis. Seven neutral microsatellite markers were characterized and genetic relatedness was calculated using Nei's genetic distance (GD) and Slatkin's RST. Among the seven DRC sites Nei's GD ranged from 0.26 to 0.92 and RST varied from -0.06 to 0.13. While genetic relatedness largely varied with distance, some geographic barriers to dispersion were noted. Parasites from the Guatemalan soldiers were closely related to parasites found in the DRC (Nei's GD=0.28) but very different from those found in Guatemala (Nei's GD=2.02). Similar results were found using Slatkin's RST (0.07 to 0.20). Clustering of isolates from soldiers and DRC was confirmed by principle coordinate analysis. The results from the study support the epidemiologic findings that the soldiers acquired malaria while they were in northeastern DRC. Molecular tools and population genetics analysis can allow us the opportunity to study the spread of malaria within and between countries. By integrating genetic and spatial information, better models of disease spread can be developed.

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EFFECTIVENESS OF MALARIA CONTROL INTERVENTIONS AMONG PREGNANT WOMEN AND CHILDREN UNDER FIVE YEARS IN A RURAL AREA OF BURKINA FASO: A RESULT FROM NOUNA HEALTH AND DEMOGRAPHIC SURVEILLANCE SITE (NHDSS) SURVEY

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Malaria remains a major cause of global morbidity and mortality, with most of the burden being in sub-Saharan Africa though Insecticide-Treated mosquito nets (ITN) have been proved to be one of the most effective intervention to prevent malaria.The 2000 Abuja summit put emphasis on promoting effective prevention methods and management of case for vulnerable groups such as pregnant women and children under five. In this study we aimed to assess the ownership and use of ITNs and access of children under five to artemisinin based combination therapy (ACT).The study took place in a rural area of north-western Burkina Faso, which was characterized as holoendemic. A cross sectional surveys were undertaken in a two samples of population derived from the Nouna Health and Demographic Surveillance Site in 2010. The first was constituted by a sample of 2850 households and the second with a sample of 409 children. Our results were compared to the Abuja indicators agreed upon by the Head of Africa States as target goals to achieve. Overall 89% of households revealed a possession of at least two bed nets among which 47.96% were insecticide treated bed nets. 24.5% of children have slept under ITNs the last night whereas it was 28.4% for pregnant women. 49.7% of children have presented a fever and 32.5% were tested positive for malaria among which 13.7% have been treated adequately with ACT. Malaria was the first cause of death (32.7%). Overall reduction of 22.8% of *falciparum* malaria prevalence was observed compared to 2006 survey data. In conclusion, many effort remains to achieve universal coverage with ITNs and clinical malaria cases management among vulnerable group so that to reach the Abuja targets and MDGs goals.

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GENDER DIFFERENCES IN INSECTICIDE TREATED NETS (ITN) USE AFTER A UNIVERSAL FREE DISTRIBUTION CAMPAIGN IN KANO STATE, NIGERIA

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The shift from targeted groups to universal coverage of Insecticide Treated Net (ITN) raises issues of gender equity and equality in access and use. There is a need for gender-based analysis to assess the effects of gender on the uptake of this key intervention for malaria control. The recent post-campaign survey in Northern Nigeria offers an opportunity to look at gender differences in ITN use. The post-campaign survey was conducted October 19-November 4, 2009 and included a random sample of 4,638 individuals in Kano State. The survey was carried out using a questionnaire adapted from the Malaria Indicator Survey. Using binary logistic regression and controlling for several covariates, we assessed the effect of gender on ITN use among all the individuals living in households with at least one ITN. ITN ownership increased more than tenfold, from 6% to 71% before and after the campaigns. There was no significant difference between the proportion of females and males living in a household with at least one ITN. However, a higher percentage of females used ITN compared to males (56% vs. 46%). After controlling for several covariates, females remained more likely to use ITNs compared to males (OR: 1.5, 95%CI: 1.3-1.7). In conclusion, this study reveals gender inequality in ITN use with men less likely to use ITN. Notably, the uptake of the intervention among the most-at-risk group (females) is higher. However, there is a need to also ensure that males equally use ITN to achieve universal coverage.

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TRANSMISSION BLOCKING EFFICACY OF ANTI-MALARIAL PLANT EXTRACTS ON *PLASMODIUM FALCIPARUM* GAMETOCYTES FIELD ISOLATES

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Targeting gametocytes, gametes and/or ookinetes; i.e. the stages of the malaria parasite responsible for its transmission from the human host to the *Anopheles* vectors, is key for pharmacological malaria control strategies. Research efforts to identify such compounds have significantly increased over the last years. However, at present, only two drugs are available, namely primaquine and artesunate, that are acting on late stage gametocytes (stage IV-V). In this study, we assessed the antiplasmodial effects of 5 extracts from 2 plants against gametocyte to ookinete stages of *Plasmodium falciparum* field isolates in an *ex vivo* assay; *Anopheles gambiae* females were membrane fed on gametocytaemic blood, treated with the plant extracts and transmission blocking activity evaluated on day 7 by assessing oocyst prevalence and density. Two of the 5 tested extracts showed significant transmission blocking activity: the commercial neem (*Azadiracta indica*) extract NeemAzal®, completely blocked oocyst development at 500, 250 and 70 µg/ml. A 90% inhibition was still found at a dosage of 50µg/ml of this seed kernel extract. Transmission blocking activity was also found with an ethyl acetate leave extract from the same plant species, inhibiting oocyst development completely at 500 µg/ml and by 80% at 250 µg/ml. The results of this study highlight the potential of anti-malarial plants for the discovery of novel transmission blocking molecules, but open also the challenging perspective of using standardized, transmission blocking herbal formulations as a complement to artemisinin combination therapy in the management of malaria and the control of the parasite's transmission.

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REDUCTION OF MALARIA PREVALENCE BY INDOOR RESIDUAL SPRAYING: A META-REGRESSION ANALYSIS

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Indoor residual spraying (IRS) has become an increasingly popular method of insecticide use for malaria control and many recent studies have reported on its effectiveness in reducing malaria burden in a single community or region. There is a need for systematic review and integration of the published literature on IRS and the contextual determining factors of its success in controlling malaria. This study reports the findings of a meta-regression analysis based on 13 published studies which were chosen from over 400 articles through a systematic search and selection process. The summary relative risk for reducing malaria prevalence was 0.38 (95% CI = 0.31-0.46) meaning a risk reduction of 62%; however, an excessive degree of heterogeneity was found between the studies. The meta-regression analysis indicates that IRS is more effective with high initial prevalence, multiple rounds of spraying, use of DDT, and in regions with a combination of *Plasmodium falciparum* and *P. vivax*.

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A NEW DEVICE FOR SURVEILLANCE AND CONTROL OF OUTDOOR BITING MOSQUITOES: ITS DESIGN, FIELD TESTING AND APPLICATIONS FOR PREVENTION OF MOSQUITO BORNE INFECTIONS IN AFRICA

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Mosquitoes that seek blood outdoors continue to contribute significantly to transmission of diseases such as malaria, filariasis and viral infections. To achieve the goals of eliminating mosquito-borne diseases, new tools that can be used outdoors are required to complement existing indoor interventions, such as insecticide-treated bed nets. We developed an odor-baited device called the Mosquito Landing Box (MLB), which can be used to control and monitor mosquitoes biting outdoors. The MLBs were baited with smelly socks and carbon dioxide gas. Field experiments were conducted in rural Tanzania to assess: *a)* the number of wild host-seeking mosquitoes visiting the MLBs, *b)* whether the mosquitoes stayed long or left shortly after arrival, *c)* the time of night when the mosquitoes were most active and *d)* whether the visiting mosquitoes could be contaminated and killed. There were significantly more mosquito vectors, *Anopheles arabiensis* (df=1, P<0.001), *An. funestus* (df=1, P<0.001), *Culex* species (P=0.028) and *Mansonia* species (P<0.001) visiting baited MLB than unbaited controls. Increasing sampling frequency from 2-hourly to either 1-hourly or half-hourly was associated with an increase in number of mosquitoes caught (df=2, P<0.002), suggesting that many mosquitoes visited the device but left shortly afterwards. Outdoor host-seeking activity was highest from 2000hours to 2200hours and from 0400hrs to 0600hrs. Adding a partially open screen-cage around the MLB did not affect catches of *An. arabiensis* (df=1, P=0.986), *An. funestus* (df=1, P=0.776) or the culicines (df=1, P>0.681). Nearly half (47.1%) of the *An. funestus* caught visiting an insecticide-treated MLBs died compared to 1.2% in controls. Further studies are underway to identify more effective and long-lasting mosquito killing agents to apply on the MLBs. These findings indicate that the MLB might be useful for sampling and possibly controlling outdoor-biting mosquitoes; by attracting, contaminating and ultimately killing the mosquitoes, hence potentially reducing disease transmission.

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VIRTUAL SCREENING FOR POTENTIAL LIGANDS OF G-PROTEIN COUPLED RECEPTORS (GPCRS): GATEWAY TO IDENTIFICATION OF NOVEL SCAFFOLDS FOR POTENTIAL TREATMENT OF NEGLECTED DISEASES

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Helmintic diseases caused by parasitic nematodes affect about 550-750 million people global. Also, malaria, caused by *Plasmodium falciparum*, was responsible for about 200 million cases and 650 000 deaths in 2010. Yet, little work has been done to discover new drugs that curb helmintic diseases. Also, the mosquito which transmits the malaria parasite has become resistant towards currently used insecticides. So, there is need to constantly search for new anti-parasitic drugs. Nematode FMRFamide-like peptides (FLPs) and the mosquito adipokinetic hormones (AKHs) alongside their G-protein coupled receptors (GPCRs) have been identified. GPCRs are known drug targets; hence intervention of the signalling pathway of FLPs and AKHs would lead to a reduction in nematode infections and the spread of malaria. Here, structure-based virtual screening of the ZINC database was performed using a previously modelled structure of a mosquito GPCR, AKHR to identify potential GPCR ligands. Docking calculations to study binding modes and affinities of a mosquito AKH (AKH-1) were done. ZINC compounds were then docked to AKHR and those with higher binding affinities for the receptor were selected for insecticidal evaluations and tested for activity towards nematode GPCRs. Docking results showed that AKH-1 had binding affinities of ΔGb =

-10.7 kcal/mol for the receptor. The AKH interacted with Tyr110, Thr129, Gln209, Lys307 and Tyr285 in the receptor binding site. From the ZINC database, about 120 compounds, containing a variety of scaffolds, showed higher binding affinities (highest affinity: ΔGb = -12.5 kcal/) for the receptor than AKH-1. These results provide novel scaffolds that could be used for potential treatment of neglected diseases.

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FINDINGS FROM A RAPID QUALITATIVE ASSESSMENT OF ACCESS TO MALARIA PREVENTION AND TREATMENT RESOURCES AMONG BURMESE MIGRANTS IN TAK PROVINCE, THAILAND

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Recent evidence of the declining efficacy of Thailand's first line artemisinin-based combination therapy (ACT), mefloquine-artesunate, in western Thailand has raised concerns that artemisinin resistance is already present on the Thai-Burma border. In Thailand, malaria occurs mainly in the border provinces, with the highest incidence occurring among Burmese migrants in Tak Province. Strategies to ensure migrants' timely access to effective malaria treatment and prevention resources are therefore of utmost importance. Although malaria case management and vector control interventions in Tak Province should be targeted to Thai nationals, refugees, and migrant workers alike, little is known about how Burmese migrants who live or work in non-refugee camp settings actually access and use malaria resources. This dearth of information on migrant malaria care-seeking behaviors has hampered Thailand's ability to assess how well malaria interventions are reaching these populations. To address this gap, in February 2012 we conducted a rapid qualitative assessment of community- and provider- level factors that affect migrants' use of malaria prevention and treatment resources in Tak Province, Thailand. Qualitative data were collected in 4 villages and 2 commercial farm settings in two districts and included 8 focus group discussions, 11 key informant interviews, 10 health provider- and 31 community- in-depth interviews, and 7 seasonal calendars. Interviews were conducted in Karen, Burmese, and Thai, digitally recorded, and transcribed in the language of interview. Interview transcripts were translated into English and content analysis conducted. Preliminary results indicate that several community and health facility factors interact to limit migrants' access to effective malaria prevention and treatment resources, particularly among non-registered migrants. These include misunderstandings about the causes of malaria, the limited availability of insecticide-treated nets for migrants, delays in reaching health facilities due to financial and legal barriers, ACT procurement and distribution delays, and language barriers between providers and their migrant patients. To minimize malaria transmission and the spread of artemisinin resistance, additional measures should be put in place to help achieve better access to malaria resources among displaced populations living in Thailand's border areas.

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STOCHASTIC MODELS FOR THE AUTODISSEMINATION OF INSECTICIDES BY MOSQUITOES

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Vector control techniques that complement indoor residual spraying (IRS) and long-lasting insecticidal nets (LLINs) may be needed to achieve malaria elimination. Semi field system (SFS) experiments were conducted to evaluate the potential for the autodissemination of pyriproxyfen (PPF) by adult mosquitoes into their breeding sites and its impact on adult mosquito emergence. We present stochastic mathematical models parameterized using SFS data to help design field trials for the autodissemination of PPF by adult mosquitoes. We incorporate stochastic characteristics of the autodissemination technique into mosquito life cycle: (1) To show how the fit between simulated and experimental data may be used to design field trials. (2) To run the model using parameters derived from mosquitoes innate life cycle characteristics such as blood-feeding, resting, flight range, and oviposition behaviour. (3) To guide field trial strategies that achieve effective insecticide coverage of breeding sites using optimal dissemination stations. Our models developed using experimental data may help design field trials for the autodissemination technique by exploiting different parameters of mosquitoes' life cycle. The model outputs suggest the design of dissemination stations, the distance between them, PPF treatment proportions and intervals necessary to achieve high coverage of breeding sites that may reduce adult mosquito density up to 40%. Mathematical models parameterized using semi-field experimental data are used to design field trials for the autodissemination of insecticides by mosquitoes.

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ISOLATION AND CHARACTERIZATION OF GRAM POSITIVE ENDOSPORE FORMING BACTERIA WITH BIO-LARVACIDAL EFFECT AGAINST MAJOR MALARIA VECTORS IN UGANDA

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Mosquito borne diseases are major causes of mortality and morbidity in the tropics. Over 20 Anophilines are of medical importance but in Uganda, *Anopheles gambiae* and *An. funestus* are the major malaria vectors. Globally, Malaria accounts for closely 1 million annual deaths, over 90% of which is from Africa. In Uganda 12.3 million cases occur with over 100,000 annual deaths and an estimated 384 child deaths per day. The comprehensive approach for malaria control includes indoor residual sprays (IRS), insecticide treated bed net distribution and malaria treatment with ACTs as well as fansidar prophylaxis in pregnant women. Despite these interventions, the malaria scourge prevails mainly due to resistance in both the parasite and the vector. There are promising results for a malaria vaccine but extensive trials are still needed before it's operationalised. Proper vector management emphasizes a multifaceted bio-rational approach where bacterial agents have been handy. *Bacillus thuringiensis* and *B. sphaericus* are the most widely studied and advanced biological agents but the spreading wave of resistance is their major setback. Their virulence has been noted to vary according to species variant and the region the agent is isolated. Therefore, there is a need to search for alternatives but none of such studies have been done in Uganda. Tree holes and water pond soil samples were pasteurized and isolates were identified microscopically for Gram and endospore reactions. They were exposed to 3rd instars of *Aedes aegypti* and mortality was observed from 3 hrs to 72 hrs. 39 isolates (9.4%) were larvacidal against *Ae. aegypti*, 80% of which were from tree holes. 1 unique isolate had terminal endospores with a drumstick appearance and produced 60% mortality within 3 hrs while the other 38 were within 24 hrs. Biochemical, molecular characterization, optimal lethal doses and *Anopheles* comparative assays are to follow. In conclusion, 39 isolates have potential of being incorporated in biological control and thus recommending their characterization and sampling from tree holes.

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BUILDING ON SUCCESS IN THE FIGHT AGAINST MALARIA: COMMUNITY INVOLVEMENT IN THE INSECTICIDE TREATED NETS HOUSEHOLD DISTRIBUTION AND HANG-UP CAMPAIGN IN GHANA

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Malaria accounted for 38.2% of Out Patient Department (OPD) attendance and 43.9% of admissions in Ghana. Insecticide treated nets (ITNs) is part of the control strategies in the country. Distribution had been through antenatal clinics with occasional mass campaigns targeting pregnant women and children under five years. Despite these efforts, ownership and usage in the country remained low. Only 33 % of households owned at least one ITN nationwide in 2008. Usage of ITNs among children and pregnant women was 28% and 20% respectively in the same year with the lowest coverage in the northern part of the country. Prominent among the reasons for the low usage was difficulty in hanging the net. To address this, a household distribution and hang up of ITNs was adopted. Community members and leaders were involved in implementing the campaign. This paper appraises the role of community members and leaders in the successful implementation of the campaign. Community leaders and chiefs supported by providing vehicles and funds to cart logistics to communities. Some chiefs and assembly members provided storage for the logistics. Chiefs and elders resolved conflicts and misunderstandings, volunteers were identified by community members and trained to move from one household to another to hang nets using their prior registration data. In some communities, members motivated these volunteers by paying a token which they had agreed on during durbars or gave them food stuff whilst in others the community leaders gave them the tokens. Through these efforts, community members received free insecticide treated nets hanged over their sleeping places. They were also taught how to maintain the net properly. In the northern region of the country six months after the campaign, household ownership of ITNs increased from 26.7% to 81.8%. ITN usage among children under five increased from 11.2% to 52.0% and from 7.0% to 39.5% in pregnant women. Community involvement is important in ensuring the success of implementation of health interventions.

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MALARIA PREVENTION MEASURES EXPENDITURES IN BURKINA FASO: HOW MUCH DO THEY COST TO HOUSEHOLDS?

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The efficacy of insecticide-treated nets (ITNs) has demonstrated in the protection from mosquito bites. The provision of insecticide-treated nets (ITNs) is widely accepted in Burkina Faso. However, other methods of prevention are used by the households. In the perspective of the introduction of the vaccine, it is important to know more about the household expenditure on the non-net products and practices. This paper aims to estimate the amounts spent on different malaria prevention measures in Burkina Faso. A cross sectional survey was conducted during the high transmission season in 2010. Simple random sampling was used to select villages and households. Data collection was carried out among 506 households in Nanoro district in Burkina Faso. Households were asked about expenditures on other forms of malaria prevention over the previous month including expenditure on coils, indoor spraying, aerosols, repellents, herbs, cleaning surrounding, environment and clearing vegetation; and any other forms of prevention. More than 50% of households used at least one malaria prevention measure. Around 98% of households owned at least one bed net. Bed nets were used by 94% of household head, 88% of under five children, 85% of husband or wife and 83% of children up to 5 years. 32% of households use smoke, 20% clean outside environment, 16% cultivate specific plants, 8% use coils, and 3% indoor spraying. The majority of households did not spend money on malaria prevention measures. Households spent an average of 2450FCFA on bed net, but only 6% of bed net were bought. Households spent an average of 910 FCFA on coils and 1768 FCFA on indoor spraying in the previous month. Household wealth was associated with coils (p=0,008), indoor spraying (p=0,04) and cleaning surrounding (p=0,009). Most of households received bed nets with the implementation of the new program of distribution of bed nets. They used more than one prevention measure. The introduction of the vaccine will affect the households' practices and it is important that it doesn't affect the utilization of the other malaria prevention practices.

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ASSESSING THE EFFICACY OF TWO TYPES OF LONG-LASTING INSECTICIDAL NETS (LLINS) TESTED IN NAMPULA PROVINCE, MOZAMBIQUE

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Long-lasting insecticidal nets (LLINs) are an important tool to prevent malaria in sub-Saharan Africa by reducing human-vector contact. In line with the new WHOPES LLIN evaluation guidelines we prospectively evaluated LLIN efficacy, including physical durability and insecticidal efficacy, on LLINs distributed during a campaign in October 2008 in Nampula Province, Mozambique. We present here the insecticidal efficacy of Olyset® and PermaNet® LLINs from the first year of follow-up. We tagged 2000 Olyset® and 4000 PermaNet® LLINs and randomly distributed them during the 2008 campaign. The tagged LLINs were then located using GPS during a house-to-house survey one month after the distribution campaign. A random sample of the households (HHs) found to have a tagged LLIN was selected for a three year follow-up evaluation. At one, two and three years after the distribution campaign, the selected HHs were surveyed and tagged LLINs were collected and transported to the Instituto Nacional de Saúde (INS) in Maputo for further evaluation. We assessed unused LLINs as the baseline. We took two day-old non blood-fed female mosquitoes from an insectary susceptible colony of *An. Arabiensis* to conduct cone bioassay tests on the baseline LLINs and LLINs collected during the first year of follow-up for both brands. Untreated polyester netting (25 cm x 25 cm) was used as negative control. A total of 49 Olyset® and 83 PermaNet® LLINs were collected during the first year of follow up. The knockdown effect was recorded after three minutes of exposure, and mortality was recorded after 24 hours post-exposure. The mortality on the baseline LLINs exposed to *An. arabiensis* was 100% for both brands. We found 96.9% and 99.7% *An. arabiensis* mortality after exposure to Olyset® and PermaNet® respectively on the LLIN collected during the first year of follow-up. Our findings show that LLINs retain their insecticidal efficacy after one year in rural Mozambique, although this starts dropping: Olyset® more rapidly compared with PermaNet® but not statistically significantly (P= 0,097). Further assessment after two and three years is on-going and results available later in 2012. These will give understanding of the long-term LLIN effectiveness in this setting.

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EFFECTS OF INSECTICIDE RESISTANCE AND CREPUSCULAR BITING BEHAVIOR ON THE COST EFFECTIVENESS OF A MASS, LONG-LASTING, INSECTICIDAL NET DISTRIBUTION: A MODELING STUDY

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The effectiveness of insecticide treated nets (ITNs) in preventing malaria is threatened by developing insecticide resistance and changing biting behavior. Data from experimental hut studies on the effects of a third generation long lasting ITN on eight anopheline mosquito populations with varying levels of insecticide resistance (from less than 10.6 % mortality in 0.05% deltamethrin WHO cylinder tests to fully susceptible) were used to parameterize malaria models. The effectiveness of a mass distribution of ITNs against malaria, in terms of episodes averted during the effective lifetime of the batch, and in terms of net health benefits expressed in disability adjusted life years (DALYs) averted, depending on resistance, biting behavior, and pre-intervention transmission level, was studied using an ensemble of 14 model variants in OpenMalaria. With the most resistant mosquito population, at the transmission level where ITNs were most effective (4 infectious bites per adult per annum (ibpapa) pre-intervention), the ITN mass distribution averted up to about 35% less episodes and DALYs than with susceptible populations. This was similar to the loss of effectiveness if 40% instead of 10% of the mosquitoes always bit during times when people are not under an ITN. Over the ranges studied, effectiveness of ITNs was more sensitive to the pre-intervention transmission level than to the level of insecticide and behavioral resistance. ITNs had positive net health benefits in most scenarios. Only at pre-intervention transmission levels above 128 ibpapa, a minority of variants of the model ensemble showed (slightly) negative net health benefits. ITNs are likely to be cost effective against malaria even in areas with strong pyrethroid resistance and where a large proportion of host-mosquito contact occurs during times when ITN users are not under their nets.

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THE ASSESSMENT OF IEC/BCC MATERIALS AND DELIVERY CHANNELS IMPLEMENTED ALONG THE THAI-CAMBODIAN BORDER

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The assessment of information, education and communication (IEC) products and communication channels was carried out in Chanthaburi and Si Sa Ket Provinces, eastern Thailand in February, 2010. The assessment was conducted to determine the acceptance, cultural appropriateness, and effectiveness of IEC materials produced to promote the behavior changes in the project "A strategy for the containment of artemisinin tolerant malaria parasites in South-East Asia". The main objective of the assessment was to understand the interim effects of IEC materials on the target audience and to find out the most effective communication channels or media to reach out to the targeted mobile and migrant population in the project. The assessment used both qualitative and quantitative methods including interviews and focus group discussions. The qualitative component helped triangulate the quantitative findings. A total of 81 respondents were interviewed in the survey. Two focus group discussions were conducted with the target audience in both provinces. The results were encouraging and showed that the coverage of IEC materials was high (80.2-83.9%) in both areas. Overall, the pamphlets were highly received among the respondents in all aspects, i.e. contents, colours, sizes, and illustrations. It was found that most of the respondents could comply with key messages appeared on the pamphlets. The findings of FGD showed similar outcomes as compared with the quantitative survey. Majority of the respondents highly accepted the posters as shown by survey. The results showed that the IEC materials were culturally appropriate and very well received by the target population. More importantly, we found that the interpersonal communication was the highly preferred communication channel for health education. These findings should be utilized as an evidence for enhancing the capacity of our field staff and volunteers to effectively deliver the health education program to the target population, as the aim set to change people's behaviors and finally to save people's health from malaria.

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FIELD EFFICACY AND PERSISTENCE OF LONG LASTING INSECTICIDE TREATED MOSQUITO NETS (LLINS) IN COMPARISON WITH CONVENTIONAL INSECTICIDE TREATED MOSQUITO NETS (ITN) AGAINST MALARIA VECTOR IN THAILAND

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In Thailand, the conventional Insecticide Treated mosquito Nets or ITN have been used over the years by the villagers. These mosquito nets are treated with permethrin 10%w/w EC manually as under the guidance of the health workers. These treated nets have efficacy for 6 months and need re-treated again. Long Lasting Insecticide treated mosquito Nets or LLINs, which can retain persistence for at least 3 years, are being considered to replace the conventional ITN. This study is intended to monitor the bioefficacy of two products of LLINs under field conditions in Thailand. These nets are PermaNet® and OlysetNet® The study was carried out in a malaria endemic area of Kanchanaburi province. PermeNet®, OlysetNet® and conventional ITN were distributed to the households were allowed to use the bed nets. The nets were washed at every 6 months intervals and only conventional ITN were re-treated after washings. WHO standard procedures for cone bioassay tests were conducted with the bed net samples collected from the households that were using the nets and laboratory reared *Anopheles* minimus were use in cone bioassay tests to access the efficiency of mosquitoes nets. Results of the study showed that both LLINs (PermaNet®and OlysetNet®) offered > 80% mortality on *Anopheles* minimus over the entire 3 years period of field evaluation. The conventional ITN performed similar to LLINs except the fact that ITN were re-treated at 6 months intervals. Interestingly the ITN offered only 15% mortality after 6 months use and were washed without re-treatment.

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ISOLATION OF ANTIPOLIOVIRUS AGENTS FROM *ZEPHYRANTHES CANDIDA* LINDL AND *CASSIA SIAMEA* LAM

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Polio eradication by vaccination of children in Nigeria has been largely unsuccessful due to the characteristic problems of accessibility, limited supervision, cultural hindrances and occasional vaccine-associated paralytic poliomyelitis. The need to consider alternative ways of managing the infection becomes imperative. This led to the ethnobotanical study of plants used for control of viral infection in South-Western Nigeria. The objective of this study was to screen for efficacy, isolate and characterize antiviral agents from these plants.Fourteen medicinal plant samples were extracted by maceration into absolute methanol at room temperature and subjected to antiviral assay. Ability of extracts to inhibit viral-induced cell death in tissue culture was evaluated three days post-infection by

MTT colorimetric assay. Linear regression was used to determine IC_{50} and CC_{50}, from which Selectivity Index (SI) was calculated. Bioassay-guided fractionation of extracts, repeated column and preparative thin layer chromatography of active fractions led to isolation of active compounds. Chemical structures of compounds were elucidated using spectroscopic techniques. The crude extracts of whole plant *Zephyranthes candida* and stem bark *Cassia siamea* were the most active of the extracts with IC_{50} of 1.85×10-3µg/mL and 1.21×10-1µg/mL respectively. Activities were retained in the chloroform fractions of *Z. candida* and *C. siamea* with IC_{50} of 1.2×10-3µg/mL and 2.3×10-1µg/mL respectively. Hexane fraction of *C. siamea* was also comparatively active with IC_{50} of 5.1×10-1µg/mL. Five compounds were isolated from *Z. candida*, namely; 7-hydroxy-3',4'-methylenedioxyflavan, lycorine, trisphaeridine, β- sitosterol glucoside and stigmasterol. Lupeol, lupenone, betulinic acid, emodin, chrysophanol, psycion and β- sitosterol glucoside were obtained from *C. siamea*. Lupeol was the most active compound from *C. siamea* with IC_{50} value of 1.4×10-2µg/mL Lycorine was the most active compound from *Z. candida* with IC_{50} value of 5.8×10-2µg/mL . Two compounds; 7-hydroxy-3', 4'-methylenedioxyflavan and trisphaeridine are reported from *Z. candida* and their anti-poliovirus properties are established. This study provides chemical entities that may be lead for development of antiviral agents.

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STUDY OF ARBOVIRUSES IN ARCHIVED SPECIMENS FROM ACUTE FEBRILE ILLNESS STUDIES IN BANDUNG, INDONESIA

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Numerous recent arboviral outbreaks have demonstrated that arthropod-borne pathogens continue to be significant public health threats. These outbreaks have not been limited to tropical or developing countries as people and goods can be moved anywhere in the world within days. Indonesia might be a very important country for emerging viruses having several endemic arboviruses including dengue, chikungunya, and Japanese encephalitis viruses. Outbreaks due to these viruses have occurred several times in Indonesia but to date studies on the existence of other arboviruses in Indonesia are scarce. Therefore, a study to detect evidence of arboviral pathogens in archived samples is currently in progress. The main purpose of this project is to identify the presence of emerging arboviruses of pandemic risk in Indonesia. We have identified archived samples from prior acute febrile infection studies that enrolled patients in two hospitals in Bandung, West Java, Indonesia from 2004-2005. The original study enrolled a total of 406 hospitalized suspect-dengue cases; the majority (311) had evidence of recent dengue infection. However, infecting etiologies on the remaining samples had not been determined. The current study includes testing for other specific endemic arboviruses as well as unknown arboviruses in the dengue negative samples. Initially, samples are tested against panels of several arboviruses including flaviviruses, alphaviruses, and bunyaviruses employing RT-PCR and IgM detection assays. Suspect positive samples are further tested with virus specific RT-PCR, viral isolation, and DNA sequencing targeting several viruses including Japanese encephalitis virus, West Nile virus, Zika virus, Chikungunya virus, Ross River virus, and hantaviruses. Our study is the first systemic survey on emerging viruses in Indonesia with gold standard molecular, serological, and virus isolation assays to estimate the magnitude of circulation of arboviruses.

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HBSAG PREVALENCE AMONG PRE-VACCINE AND VACCINE ERA CHILDREN IN BANGLADESH: PRELIMINARY RESULTS

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Hepatitis B virus infection is a leading cause of morbidity and mortality due to hepatocellular carcinoma and liver cirrhosis worldwide. Some previous small scale studies in Bangladesh observed hepatitis B surface antigen (HBsAg) prevalence as 3%-7%. During 2003-2005 Bangladesh introduced hepatitis B (HepB) vaccine into the routine childhood vaccination schedule provided at 6, 10 and 14 weeks of age. While a birth dose of HepB vaccine is widely recommended, the country's high prevalence of home births (85%) presents logistical difficulties to timely administration of a birth dose. This study evaluated the impact of HepB vaccine introduction in Bangladesh in the absence of a birth dose by comparing HBsAg prevalence among children born before and children born after HepB introduction. Using probability proportional to size cluster sampling, we selected a nationally representative sample of 2,100 pre-vaccine era children born from April 1, 2001 to March 31, 2002 and 2,100 vaccine-era children born from November 1, 2005 to October 31, 2006 from 105 clusters. In each cluster, starting from the center to a randomly chosen direction, we visited all households until we found 20 pre-vaccine era and 20 vaccine-era children. After taking written consent from their guardians, we collected a blood sample from each child along with vaccination and demographic information. We performed a rapid test of HBsAg in the field (Abbot Determine; sensitivity: 95%-100% and specificity: 96%-100%). Confirmatory testing will use standard serologic assays. To enroll 2,100 children from each group, we approached 2,203 pre-vaccine era children (refusal rate 5%) and 2,270 vaccine-era children (refusal rate 8%). Among the enrollees, 97% of vaccine-era children received HepB from national childhood vaccination program and 2.7% of pre-vaccine era children received HepB from private sources. None of 2,100 vaccine-era children were HBsAg-positive; by comparison, 24 (1.1%; 95% CI=0.7-1.7) of 2,100 pre-vaccine era children were HBsAg positive. Preliminary results suggest that even without a birth dose, the HepB vaccine program was highly effective in Bangladesh. Considering the long term efficacy of HepB, childhood HepB vaccination will continue to reduce the national hepatitis B burden. These findings support continued investment in HepB in other countries who introduced HepB vaccine into childhood immunization programs but have not yet evaluated impact.

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SEROPREVALENCE OF CYTOMEGALOVIRUS INFECTION IN A COHORT OF CHILDREN EXPERIENCING DIFFERENTIAL MALARIA TRANSMISSION DYNAMICS IN WESTERN KENYA

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Cytomegalovirus infection is a serious cause of congenital disease in western countries where it causes sensoneural hearing loss and neurodevelopmental disorders and is one of the main viruses associated with transfusion related infections. The virus can be reactivated and causes fatal infection in immunosuppressed individuals. The prevalence of CMV infection in American adults is approximately 54% while approximately 85% of Gambian children acquire CMV infection by their first birthday. However, little is known about the prevalence of CMV in Kenyan children.

Our aim was to determine the CMV seroprevalence in a cohort of children from western Kenya. Infants were enrolled from two rural sites in Western Kenya: Kisumu District where malaria transmission is holoendemic and Nandi District where malaria transmission is sporadic. Blood samples from infants born to HIV-seronegative mothers were taken from 1 month through 24 months of age to measure CMV viral load in peripheral blood and CMV antibodies. CMV-specific IgG was assessed using a luminex bead based array assay and viral loads were measured in DNA extracted from whole blood using quantitative PCR. Preliminary results shows that CMV seroprevalence increases with age with a seroprevalence of 60% versus 22% at three months, 80% versus 52% at 6 months, 91% versus 83% at 12 months and 100% versus 92% at 24 months in malaria holoendemic and sporadic areas respectively. Studies are ongoing to analyze CMV IgM to confirm whether there is primary infection by 3 or 6 months of age. These preliminary results show higher seroprevalence of CMV in infants living in a malaria endemic area within the first year of life compared to infants from a region with sporadic malaria transmission. Further studies are needed to understand why seroprevalence of CMV in a malaria endemic region is so high early in infancy.

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A SURVEY OF ARBOVIRUSES CIRCULATING IN KENYA 2007-2010

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Arbovirus surveillance in mosquitoes during the inter-epidemic period of 2007-2010 in six sites in Kenya indicated that several viruses were in circulation. The sites Garissa ,Magadi ,Turkana , Tanadelta , Budalangi and Naivasha are well spread across the country .A total of 25 isolates were made comprising of 4 viruses from three arbovirus families.11 of the isolates have not yet been fully characterized. The mosquitoes were collected using CO_2-baited CDC light traps from December 2009 to June 2010 in the selected sites during wet seasons. Mosquitoes were identified to specie and pooled (≤ 25 mosquitoes per pool).Consequently, the mosquitoes were homogenized in 1.5ml eppendorf tubes containing one 4.5mm copper bead and 750µl of minimum essential medium supplemented with 15% FBS, 2% L-Glutamine and 2% Antibiotics mixture. The homogenates were centrifuged at 10,000 rpm at 4^0C for 15min and the resultant supernatants inoculated in monolayers of VERO cells in 24 well plates. The cultures were incubated at 37˚C and monitored for cytopathogenic effects (CPE) daily upto 14 days. Cultures showing CPE were harvested and viruses identified by RT-PCR and sequencing. Bunyavirus isolates were obtained from pools of *Aedes mcintoshi* (4), *Anopholes funestus* (3) and *Ae. tricholabis* (1).Five of these mosquitoes were collected from Garissa, two from Magadi and One from Tanadelta. Alphaviruses were isolated from pools of *Cx. univittatus(3), Culex spp(1)* , *Ae. luridus* pools(2) , *Ae. tricholabis*(2), *Ae. mcintoshi(2) and Aedes spp*(1). Six of these isolates were collected Garissa ,two from Naivasha ,one from Budalangi and three from Tanadelta .All Flavivirus isolates were isolated from *Cx.univittatus* mosquitoes ,five of which were from Garissa and one from Turkana. Eight Bunyaviruses (4 Bunyamwera, 1 Pongola), 11 Alphaviruses (4 Ndumu, 3 Babanki) and 6 Flaviviruses (6 West Nile viruses) were isolated. Four of the Alphaviruses and three of the Bunyaviruses have not yet been fully characterized.This results indicate that continued arbovirus surveillance in this region is important to avert future arbovirus outbreaks,map out risk zones and direct correct diagnosis of febrile illnesses in the human population.

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SEROLOGICAL EVIDENCE OF NIPAH LIKE VIRAL INFECTION IN PIGS IN BANGLADESH

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Nipah virus (NiV) causes fatal encephalitis in humans; Pteropus bats are their natural reservoir. Pigs may act as a potential amplifier of NiV and transmit the disease to humans as in Malaysia 1998-99. We looked for serological evidence of NiV infection in pigs in three North-western Districts in Bangladesh where human NiV infections were repeatedly identified. From May to September 2009 we collected blood samples from 312 pigs (*Sas scrofa*) (Rajshahi, n=100; Nawabganj, n=102; and Naogaon, n=110) from backyard and nomadic herds over six months of age. We tested the serum samples for antibody binding to a NiV recombinant G glycoprotein using a Luminex assay. Samples having median fluorescence intensity over 1000 were considered positive. Samples tested positive for antibody were further tested by serum neutralization test against the NiV N protein. To understand the difference between exposure (age, sex, pig raising pattern) and outcome (NiV antibody) we performed Wilcoxon rank-sum test and chi-squared. Of the 312 pigs, 60 had antibody against NiV G protein by luminex testing [19%, 95% Confidence Interval (CI): 14 - 24]. However, none of the serum samples demonstrated serum neutralization. The prevalence of NiV G protein antibody in pigs was higher in Rajshahi 26% [95% CI 17 - 38] and Nawabganj 23% [95% CI 16 - 33] than in Naogaon district 9% [95% CI 4 - 16]. Compared with the pigs that lacked NiV G antibody, pigs with NiV G antibody did not differ by age (median 23 months vs. 22.8 months, P=0.9); sex (21% female vs. 18% male, P=0.5); and raising pattern (pigs with NiV antibody: 20% raised in backyard vs. 15% raised in nomadic herds, P=0.4). In conclusion, this study identified serological evidence of Nipah or perhaps a closely related virus infecting pigs in Bangladesh. The difference in prevalence by geography suggests that the positive antibody tests did not result from nonspecific binding with porcine antigens. Actively screening pigs to identify henipavirus infections, may identify viruses of public health importance.

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SEROLOGICAL AND MOLECULAR CHARACTERIZATION OF SUSPECTED CASES OF HEMORRHAGIC FEVER AND HEPATITIS VIRUSES IN NORTHERN GHANA

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Haemorrhagic Fever (HF) viruses are prevalent in West Africa and have led to outbreaks with considerable morbidity and mortality. Evidence abounds for HFs but the causative agents are not fully discerned. Molecular and serological tools to diagnose Lassa fever, Yellow fever and other viral haemorrhagic fevers (VHFs) and research programmes identifying VHF agents, as well as estimating their public health relevance rarely exist in Ghana. Reports on the prevalence ratio of viral hepatitis (VH) in Ghana is one in six and this was consistent with a recent data in a study. There are no guidelines for the screening of viral markers for viral hepatitis; testing is left to the local health institutions to implement based on high index of suspicion. Routine screening for HAV and/or HEV are not

performed. However, reported fatal cases of HEV in pregnant women in southern Ghana have been documented. This study sought to establish the prevalence of VHFs and VH in Northern Ghana. Base on reports and geographical locations of border countries with confirmed VHF cases, 16 health facilities were chosen as sentinel sites. Patient consenting and meeting the case definition were recruited and sampled. Virus detection and characterization by serological and molecular techniques was done for viral agents associated with VHF. Laboratory analyses were conducted on 276 serum samples. Investigations with RT-PCR assays for all the clinical specimens were found negative for VHF virus types, Lassa, Crimean Congo, Yellow fever, Dengue, Ebola, Marburg, and Rift Valley. Anti-Lassa fever IgG antibody titers were recorded for one case (titer ≥ 1:20) and 8 cases of anti-Dengue type-2 IgG (titer ≥ 1:80). Two cases exhibiting specific IgG (titers 1:1280 and 1:1280) and IgM (titers 1:20 and 1:20) against Chikungunya virus were found. Viral nucleic acid were however detected for viral hepatitis agents including; 21 (7.6%) for Hepatitis A; 58 (21.0%) for Hepatitis B, and 23 (8.3%) for Hepatitis C viruses. Anti-HEV IgM antibodies in all serum samples evaluated were 62 (22.5%). Our findings do not indicate a significant presence of VHF agents in Northern Ghana. However, the data generated suggest that VH infections, which often share clinical symptoms with VHFs are widespread, illustrating the need for differential diagnosis to be implemented.

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NON-POLIO HUMAN ENTEROVIRUSES ASSOCIATED WITH RESPIRATORY INFECTIONS IN PERU (2005-2010)

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Human enteroviruses (HEVs) are known to cause respiratory tract infections and are classified into four groups (A-D) and 106 different serotypes. Little is known about the various HEVs' role in respiratory infections in South America. This study describes the epidemiology and phylogenetic characterization of non-polio HEV respiratory infections in patients with influenza-like illness enrolled in a passive surveillance network in various regions of Peru. Oropharyngeal swabs and epidemiological data were collected from participants after obtaining verbal consent. Viral isolation was performed in cell culture and identified by immunofluorescence assay. Serotype identification of HEV isolations was performed using commercial monoclonal antibodies. Identification of non-serotypeable isolations was carried out by reverse transcriptase-PCR, followed by sequencing for genotype determination. Between 2005 and 2010, we analyzed a total of 24,240 samples. We identified at least one respiratory virus in 41.1% of samples (9971/24240); of those, HEV was found in 173, for a prevalence of 0.7% (173/24240). Our results revealed a clear predominance of HEV-B species, 92.5% (160/173). No isolations of HEV-C and HEV-D were found. The mean age and standard deviation for HEV-positive subjects were 9.2 and 12.5 years, respectively, much lower than the population sampled (17.6 and 17.5 years, respectively). A total of 15 different serotypes were identified, with the most common being coxsackievirus B1, coxsackievirus B2, coxsackievirus A16, and enterovirus 71. This study is the first to report HEV isolation from respiratory specimens in Peru. Compared with other countries in South America, our HEV prevalence in ILI was similar to those from Ecuador and Brazil, and our HEV-group breakdown was similar to that found in Brazil.

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SEROLOGICAL CHARACTERIZATION OF GROUP C VIRUSES ASSOCIATED WITH FEBRILE ILLNESS IN PERU, 1999 - 2011

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Group C viruses are arthropod-borne viruses with tri-segmented RNA genomes belonging to the genus *Orthobunyavirus*, family *Bunyaviridae*. Group C viruses were first identified near Belem, Brazil, in 1954 and have since been found across tropical and subtropical areas of the Americas. Group C viruses are common causes of sporadic, mild to severe, self-limiting febrile illnesses characterized by headache, vertigo, backache, muscle and joint pain, nausea, and photophobia. While Group C viruses have not been associated with epidemics or fatal infections, orthobunyaviruses have been shown to have potential for emergence in humans and livestock, underscoring the importance of understanding their epidemiology and ecology. To address this need, we conducted a serological characterization of Group C viruses isolated from febrile patients in Peru between 1999 and 2011. Patients with fever >38°C and signs and symptoms compatible with viral infection were enrolled in a clinic-based febrile surveillance study in sites across Peru (Cusco, Junin, Lima, Loreto, Madre de Dios, Piura, and Tumbes). The identification of Group C viruses was made initially by viral isolation using Group C hyperimmune ascitic fluid in an indirect immunofluorescence test. Microneutralization tests, considered the most specific serological assay available, were used to distinguish among closely related Group C viruses. Between 1999 and 2011, 61 Group C isolates were recovered from approximately 32,000 acute-phase febrile serum samples. All of the Group C viruses identified were isolated from participants in the jungle cities of Iquitos, Yurimaguas, and Puerto Maldonado. Isolates serologically consistent with Caraparu virus (62.3%), Murututucu /Marituba (34.4%) and Itaqui virus (3.3%) were identified. These results demonstrate the sustained transmission of and human exposure to distinct Group C viruses across Peru. Serological data will be paired with full-genome sequences to further explore the evolutionary relationships among Group C viral strains.

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FIELD TRIAL FOR RIFT VALLEY FEVER CLONE 13 VACCINE IN LIVESTOCK FARMS IN KENYA

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We carried out a field trial of Rift Valley Fever (RVF) Clone 13 vaccine, produced by Onderstepoort Biological Products, South Africa, to determine its safety and efficacy in commercial livestock in Kenya. Experimental studies have suggested that Clone 13 is a superior RVF vaccine when compared to the currently available Smithburn strain vaccine, in terms of safety and efficacy, and Clone 13 has the potential for testing that can differentiate infected and vaccinated animals (DIVA). General health observations, injection site reactions, and rectal temperature were assessed by monitoring all animals for the first 3 days and on day 14 post-vaccination. Blood specimens were obtained on days 0, 14, 28, 56, 183 and 366 after vaccination and sera tested for the presence of IgM and IgG antibodies against RVF virus. The individual farm animal husbandry and reproduction management system was maintained for the study animals. A total of 404 animals (cattle, sheep, goats) located at three commercial farms in Kenya were enrolled in the study. Of these, 170 (42.1%) animals were not pregnant whereas 234 (57.9%) were at either early or late pregnancy. Of the 404 animals, 195(48.3%) were inoculated with RVF Clone 13 vaccine, and the remaining 209 (51.7%) animals inoculated with saline as placebo. Eleven (2.7%) study animals were lost to follow up after being sold following accidental trauma or lost to predation. Most animals maintained good health following vaccination with no adverse events except for 20 sheep (4.9%) that developed

uncomplicated mild nasal discharge 1 to 3 days post vaccination confirmed as bacterial infections; all recovered. One cow was treated for placenta after birth. Of 234 pregnant animals that delivered, 231 (98.7%) had live births including 31 cattle, 102 sheep (including 10 twins), and 98 goats (including 26 twins); however, one (0.4%) abortion and two (0.8%) still births occurred in goats. No teratogenicity was observed in any offsprings. Both IgM and IgG antibodies were detected in vaccinated but not unvaccinated animals. In conclusion, the RVF Clone 13 vaccine was found to be safe for use in cattle, sheep, and goats including animals in early and late pregnancies. The vaccine also appears to have good efficacy as demonstrated by detection of antibodies against the virus.

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IDENTIFICATION OF A NOVEL ORTHOBUNYAVIRUS ISOLATED FROM *CULEX (MELANOCONION) PORTESI* MOSQUITOES FROM IQUITOS, PERU

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Vector-borne pathogens are among the most important emerging and re-emerging viruses that cause epidemics in humans and livestock. In this study, we describe the isolation and molecular characterization of a novel orthobunyavirus (genus *Orthobunyavirus*, family *Bunyaviridae*) isolated from *Culex (Melanoconion) portesi* during entomological surveillance for arboviruses in the city of Iquitos, located in the Amazon basin of northeastern Peru. Mosquitoes were collected by CO_2-baited CDC light traps, identified to species, pooled, triturated, and inoculated onto mosquito (C6/36) and mammalian (Vero 76) cell cultures. In one pool of *Culex (Mel) portesi* collected in November 2009, evidence for orthobunyavirus infection was detected by cytopathic effect and indirect immunofluorescent assay in inoculated Vero 76 cells using polyclonal antibodies against Group C orthobunyaviruses. Hyperimmune ascitic fluid against a range of Group C viruses, including Apeu, Itaqui, Murututucu, Marituba, Oriboca, and Caraparu viruses, were unable to neutralize the virus isolate in microneutralization assays. Full genome sequence was generated by random amplification and pyrosequencing and compared with sequences available in GenBank. While the highest sequence similarity was with Group C viruses, nucleotide (<75%) and amino acid identity (<70%) was low for S, M, and L segments compared with previously reported viruses. Based on serological and molecular results, we conclude that this isolate is a novel member of the Group C orthobunyaviruses. Future studies will be necessary to determine the prevalence and possible human health impact of this newly-identified virus.

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IMMUNE RESTORATION DISEASE AND CHANGES IN CD4+ T-CELL COUNT IN HIV-INFECTED PATIENTS DURING HIGHLY ACTIVE ANTIRETROVIRAL THERAPY AT ZEWDITU MEMORIAL HOSPITAL, ADDIS ABABA, ETHIOPIA

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Highly active antiretroviral therapy (HAART) improves the immune function and decreases morbidity, mortality and opportunistic infections (OIs) in HIV-infected patients. However, since the use of HAART, immune restoration disease (IRD) has been described in association with many OIs. Our objective was to determine the proportion of IRD, changes in CD4+ T-cell count and possible risk factors of IRD in HIV-infected patients. A retrospective study of all HIV- infected patients starting HAART between September 1, 2005 and August 31, 2006 at Zewditu memorial hospital HIV clinic, Addis Ababa, Ethiopia was conducted. All laboratory and clinical data were extracted from computerized clinic records and patient charts. A total of 1166 HIV- infected patients with mean ± SD age of 36 ± 9.3 years were on HAART. IRD was identified in 170 (14.6%) patients. OIs diagnosed in the IRD patients were tuberculosis (66.5%, 113/170), toxoplasmosis (12.9%, 22/170), herpes zoster rash (12.9%, 22/170), *Pneumocystis jirovecii* pneumonia (4.1%, 7/170), and cryptococcosis (3.5%, 6/170). Of the 170 patients with IRD, 124 (72.9%) patients developed IRD within the first 3 months of HAART initiation. Low baseline CD4+ T-cell count (odds ratio [OR], 3.16, 95% confidence interval [CI], 2.19-4.58) and baseline extra pulmonary tuberculosis (OR, 7.7, 95% CI, 3.36-17.65) were associated with development of IRD. Twenty nine (17.1%) of the IRD patients needed to use systemic anti-inflammatory treatment where as 19(11.2%) patients required hospitalization associated to the IRD occurrence. There was a total of 8 (4.7%) deaths attributable to IRD. In conclusion, the proportion and risk factors of IRD and the pattern of OIs mirrored reports from other countries. Close monitoring of patients during the first three months of HAART initiation is important to minimize clinical deterioration related to IRD.

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DISTRIBUTION OF KILLER CELL IMMUNOGLOBULIN-LIKE RECEPTORS (KIR) GENES IN AN ADMIXED PERUVIAN POPULATION

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Killer cell immunoglobulin-like receptors (KIR) are glycoproteins located on the surface of NK cells. These receptors are classified into two groups according to their cytoplasmic domain, which transduces inhibitory or activating signals, and consequently modulates NK cell function and most likely the susceptibility to diseases or infections. We studied the distribution of KIR genes in 363 Peruvian HTLV-1-infected individuals using two ethnic classification methods: 1) a questionnaire, which defined the participants as Andean (both parents born in the Andes) or Mestizo (only one parent born in the Andes); and b) ancestry informative markers (AIM), which allowed classifying the whole population into three groups according to their ethnic admixture proportions. DNA was obtained from blood samples of each individual and KIR genotyping was carried out using PCR-SSP. No significant differences were observed in gender and age according to the Andean/Mestizo classification, whereas significant differences were found when the ethnic admixture proportion criterion was applied. The frequency of KIR2DS3, KIR2DS4 and KIR2DL3 were statistically different between Andeans and Mestizos. When using ethnic admixture proportion, significant differences were observed for KIR3DL1 and KIR2DS4s in addition to those genes, among the three groups defined. No significant differences were detected in haplotypes and inhibitory-activating KIR genes using either the questionnaire or AIM-based classification. AIM helps minimizing both the bias in ethnic group definition and the effects of population stratification, and therefore should be used in order to avoid false results when searching for gene-disease associations in admixed populations.

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SEROPREVALENCE OF SELECTED ARBOVIRUSES IN IJARA AND MARIGAT DISTRICTS, KENYA

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Arboviruses are transmitted by arthropods; humans become infected during blood meal by infected mosquitoes, ticks and sandflies. Arboviruses have been well characterized in many industrialized countries, but there are many knowledge gaps in developing nations. Entomological surveys conducted so far have demonstrated circulation of arboviruses of significant public health importance in *Aedes, Anopheles* and *Culex* species in vast populations in Kenya, suggesting the presence of competent vector systems for many of the arboviruses. The human population involvement in the transmission of these viruses has however not been demonstrated. This study sought to determine the sero-prevalence of a range of arboviruses including Chikungunya, Dengue, Sindbis, Sandfly Naples, Sandfly Sicilian, Uganda S, West Nile and Zika viruses in Ijara and Marigat. About 5µl of patients' serum samples was used to test for antibodies to each of the viruses listed. All the samples were tested by IgG ELISA.A total of 351 patient serum samples were analyzed, of these 193 (54.9%) were male while female were 158 (45.1%), and age range was between 3 and 73. The overall positivity for the arboviruses was 53/351 (15.1%). The arboviruses prevalence in Marigat was 7% (10/143) while Ijara was 21% (43/208). Uganda S virus was the most prevalent with 10%, followed by West Nile virus 6%, Sindbis 5%, Dengue 2%, Chikungunya 1.1%, Sandfly Naples 0.2%. Antibodies against Sandfly Sicilian and Zika viruses were not detected. This is the first documentation of antibodies against Sandfly Naples virus in the sub-Sahara Africa. This study has shown the evidence of past exposure of the selected arboviruses in human population in the two sites. This information together with vectors data will strengthen efforts to develop focused preventive actions to stop transmission and create awareness among clinicians to help improve patients' management in the region.

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LONG-TERM CLINICAL, IMMUNOLOGIC AND VIROLOGIC FOLLOW-UP IN A COHORT INFECTED WITH MAYARO VIRUS

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Alphaviruses, such as Venezuelan equine encephalitis virus and chikungunya virus, are mosquito-borne pathogens possessing the potential of causing explosive epidemics with chronic sequelae in those infected. Much less is known about Mayaro virus (MAYV), an alphavirus endemic to the Amazon basin that causes fever, malaise, and joint pain in the acute setting. Long-term studies of this virus are rare and limited to case reports. Starting in January 2011, as part of a collaborative febrile surveillance project with the Peruvian Ministry of Health in the Amazon cities of Iquitos and Yurimaguas, we enrolled 17 patients with acute MAYV infection confirmed by isolation (11), PCR (6), or ≥ 4-fold change in IgM titer (17)_most patients had more than one positive assay. In addition to their acute visit, patients were also evaluated at 20 days (±10 days), 3 months (±10 days), and 6 months (±15 days). At each follow-up visit, a detailed interim history was taken and a physical examination was performed. In addition, serum was obtained in order to evaluate IgM and IgG titers and urine was collected and will be evaluated with PCR. Preliminary results show that while IgG levels persist at high levels at 6 months, IgM titers remain elevated in most patients at 3 months, but return to zero in the majority at 6 months. Fourteen of seventeen patients had arthralgias at the acute visit, 3 of 17 (18%) at 20 days, 7 of 16 (44%) at 3 months, and 6 of 12 (50%) at 6 months. Distal joints, specifically of the hand and ankle, were the most commonly affected. Malaise, present in 17 of 17 (100%) at the acute visit, persisted in 2 of 17 (12%) patients at 20 days, 2 of 16 (13%) at 3 months, and 0 of 12 (0%) at 6 months. Our investigation will continue to follow these patients at one and two year visits, and we will also investigate immunologic and virologic findings that correlate with long-term morbidity.

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POST-EPIDEMIC SEROPREVALENCE OF RIFT VALLEY FEVER VIRUS AMONG SOMALI VILLAGES IN NORTHEASTERN KENYA

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In endemic areas, Rift Valley fever virus (RVFV) is a significant threat to both human and animal health. Goals of this study were to measure human anti-RVFV seroprevalence in a high-risk area following the 2006-2007 Kenyan Rift Valley Fever (RVF) epidemic, to identify risk factors for RVFV exposure, and to monitor for sequelae of RVFV disease. We conducted a large cross-sectional village cluster survey among residents aged 1-85 years in 6 villages in Ijara District, Northeastern Province, Kenya: Tumtish (N=190, 47 households), Matarba (N=242, 70 households), Korahindi (N=289, 86 households), Gedilun (N=237, 63 households), Golalbele (N=85, 27 households), and Sabenale (N=64, 20 households). Participants underwent questionnaire administration, physical exam, vision testing, and blood collection for RVFV testing. One thousand one hundred seven individuals were tested for RVFV exposure via RVFV IgG ELISA; 667 or 60% were women and 631 or 57% were children aged 1- 15 years. Overall, 173/1111 or 16% (CI95% 13.78-18.42) of local residents were RVFV seropositive. Seroprevalence varied by village: Tumtish (27/190, 14%, CI95% 9.59-20.02), Matarba (37/242, 15%, CI95% 11.00-20.27), Korahindi (53/291, 18%, CI95% 15.63-24.99), Gedilun (32/237, 13.5%, CI95% 9.27-18.05), Golalbele (15/87, 17%, CI95% 9.98-26.84), and Sabenale (10/64, 15.6%, CI95% 7.76-26.86). Visual impairment (defined as ≤20/20) was much more likely in the RVFV-seropositive group (P= 0.0001). Our results highlight significant variability in RVFV exposure in six neighboring villages having very similar climate, terrain, and Somali populations. In concordance with previous studies, RVFV seropositivity was associated with poor visual acuity. Further analysis of questionnaire data will elucidate primary risk factors for RVFV exposure.

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REVISITING ALBERT SABIN'S RESEARCH ON HUMAN DENGUE INFECTION

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There is no good animal model for dengue, one of the most important emerging tropical infectious diseases. As a result, we have relied on

human studies done by the US Army in the 1920s and 1940s for basic information on incubation period, cross protective immunity and early clinical presentation. One study was conducted by Albert Sabin, who infected 118 volunteers with DENV-1 and -2 isolated in Hawaii, New Guinea and India. Sabin presented a broad overview of these studies in his oft-cited 1952 paper, but the details were never published. His original laboratory records, which were bequeathed to one of us (DJG), provided the opportunity to re-visit these seminal studies. Of 118 primary infections (104 DENV-1 and 14 DENV-2), 20 subjects were reinfected with the heterologous serotype at intervals of 1.5 - 9 months to evaluate the duration of cross-protective immunity between serotypes. Median fever duration for primary DENV-2 infections was 2.2 days compared to 3.3 days for primary DENV-1 ($p < 0.05$), although maximum temperature and severity of illness were comparable. Of 7 secondary infections at 6-8 weeks, all but one had temperature <38°C; one with a maximum temperature of 38°C on day 8, was viremic. At 8-10 weeks post primary infection, 4 out of 5 (80%) subjects became ill with maximum temperature between 38.1 and 38.8°C. At 4-9 months post primary infection, 7 out of 8 (88%) subjects with secondary infection developed fever and 3 (38%) had a maximum temperature >39°C. There was a trend towards a shorter incubation period in secondary infections at 4-9 months (median 4.2 days) compared to primary infections (median 6.9 days), and illness was generally milder in secondary infections. The data suggest that the DENV serotype and/or strain may influence the duration of fever in primary infection, and the time elapsed from the primary infection influences the symptoms, duration and severity of fever and leukopenia in secondary infections. A detailed analysis of the Sabin experiments will be presented.

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DENGUE VIRUS INFECTION IN THE SKIN: DENDRITIC CELLS AND THE ROLE OF MOSQUITO SALIVA

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Dendritic cells (DCs) that reside in the skin serve as sentinels of the immune system. The 4 dengue virus serotypes (DENV1-4) are transmitted when infected *Aedes aegypti* or *Ae. albopictus* mosquitoes bite humans, and may result in dengue, the most prevalent arthropod-borne human viral disease. Primary DENV infection confers life-long protection to the same serotype, while secondary infection with a distinct DENV serotype is a major risk factor for severe disease via serotype cross-reactive enhancing antibodies (ADE) or T cells. DCs, monocytes, and macrophages are targets of DENV in humans, yet little is known about initial DENV infection in the skin after the bite of an infected mosquito or about subsequent systemic viral spread. Here, we report a novel intradermal (i.d.) infection model with DENV2 strain D220 in the ears of C57BL/6 mice deficient in the IFN-α/β receptor ($IFNAR^{-/-}$), under primary and ADE infection conditions. Ear skin is digested to separate epidermal from dermal sheets and to prepare single cell suspensions, which are stained with monoclonal antibodies (MAbs) against surface markers to distinguish cell populations and MAbs against intracellular DENV nonstructural protein 3 and envelope to identify DENV-infected cells by flow cytometry. 24 and 48 hours after infection, replicating DENV was detected in epidermal Langerhans cells, dermal $CD11b^+$ DC and $CD103^+$ DC subsets, and $CD45^-$ non-hematopoietic cells in both the epidermis and dermis. Inoculation of sub-neutralizing levels of DENV-specific MAbs resulted in increased morbidity and mortality of $IFNAR^{-/-}$ mice after i.d. DENV infection; we are now analyzing DENV-infected skin cell populations under ADE conditions. While probing for blood vessels, mosquitoes eject saliva, which contains pharmacologically active substances, into the skin. When mice were pre-sensitized to salivary gland extracts (SGE) from female *Ae. aegypti* mosquitoes by repeated i.d. inoculation of SGE 4 and 2 weeks prior to co-injection of DENV2 D220 and SGE, preliminary results revealed reduced numbers of $DENV^+$ cells in the dermis compared to naïve mice that were not pre-sensitized to SGE. We are currently expanding the investigation of the role of vector saliva and ADE on DENV replication in the skin, spread of infection, and induction of the immune response. These findings will improve understanding of the initial events following DENV infection and will inform future vaccine development.

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ANALYSIS OF EARLY DENGUE VIRAL INFECTION IN MICE AS MODULATED BY *AEDES AEGYPTI* PROBING

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Mosquito saliva contains proteins with anti-inflammatory, anti-hemostatic, and immuno-modulatory capabilities. Arbovirus-infected mosquitoes expectorate saliva and virus immediately prior to blood feeding and this saliva has been shown to aid the establishment of arboviruses such as Sindbis and West Nile within the vertebrate host. Limited work exists on the functional relationship between *Aedes aegypti* salivary proteins and vertebrate infection with dengue virus. Most studies have focused on associations between disease severity and changes observed in the early days post exposure rather than interactions that could affect establishment of dengue virus within a vertebrate host. Here, we examine *in vivo* transcriptional changes of critical innate immune pathways three hours post exposure at inoculation sites of IRF3/7 -/--/- (C57BL/6) mice. Mice received dengue serotype 2, strain 1232 intradermal needle-inoculation where field collected *Ae. aegypti* mosquitoes 1) had and 2) had not recently probed. At inoculation sites where mosquitoes had probed, we observed a generalized up-regulation of the transcripts for transcription factors, such as relA, and transcription factor associated proteins. We found substantial down-regulation of various cytokines including the interferon γ transcript and the transcript of a monocyte chemotactic molecule, IP-10. Additionally, we found an approximately 45-fold down-regulation of the transcript for toll-like receptor 7, an endosomal pattern recognition receptor for single-stranded RNA. The down-regulation of these transcripts early in the infection process could indicate important mechanisms by which mosquito salivary proteins serve to enhance the establishment of dengue viral infections in the vertebrate host.

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DENGUE VIRUS-SPECIFIC HUMORAL AND T CELL RESPONSES IN NOVEL HUMANIZED MICE

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Dengue is a mosquito borne viral disease of humans, and animal models that recapitulate human immune responses and/or dengue pathogenesis are needed to understand the pathogenesis of the disease. We recently described an animal model for dengue virus (DENV) infection using humanized NOD-*scid IL2rγ*^{null} mice (NSG) engrafted with cord blood hematopoietic stem cells (HSC). We sought to further improve this model by co-transplantation of human fetal thymus and liver tissues into NSG (BLT-NSG) mice. Enhanced DENV-specific antibody titers were found in the sera of BLT-NSG mice compared to human cord blood HSC-engrafted NSG mice. Furthermore, B cells generated during the acute phase and in memory from splenocytes of immunized BLT-NSG mice secreted DENV-specific IgM antibodies with neutralizing activity. We have generated and characterized a panel of human monoclonal antibodies (MAbs) from B cells in BLT-NSG mice during acute DENV infection and in convalescence. Human T cells in engrafted BLT-NSG mice secreted IFN-gamma in response to overlapping DENV peptide pools and HLA-A2 restricted peptides.

BLT-NSG mice will provide a much-needed platform to assess human immune responses to DENV vaccines and the effects of prior immunity on subsequent DENV infections.

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TRANSMISSION OF DENGUE TO MOSQUITOES DURING PERIODS OF LOW VIREMIA

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Our understanding of dengue transmission dynamics is largely driven by hospital and/or symptomatic case data. It has been suggested that higher viremia levels, as observed in patients with more severe disease, may result in a higher probability of uptake by a vector, thus enhancing transmission. Comparatively little attention has been paid to the role of lower level viremic and possibly asymptomatic cases in dengue transmission. Such inapparent infections may account for the majority of dengue infections. Additionally, as dengue transmission occurs at a highly focally spatial level (households, schools, workplaces), the transmission to mosquitoes from such low viremias has not been sufficiently investigated. Accordingly, we investigated the rate of acquisition, subsequent dissemination and transmission of dengue virus serotype 2, strain 1232 at low viremia levels by *Aedes aegypti* (Rockefeller) mosquitoes from a permissive mouse model. Cohorts of mosquitoes kept for up to fifteen days post exposure to viremic mice were allowed to refeed on naïve mice, and were then tested for virus infection and dissemination. Critically, at viremias as low as $1x10^1$ and $1x10^2$ pfu/ml, mosquitoes acquired virus infections, with predicted transmission rates of 7-18%. Our findings suggest that during lower viremia levels, asymptomatic (or prepatent) cases may account for an important proportion of the transmission potential to mosquito vectors.

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GENOME-WIDE PATTERNS OF INTRA-HUMAN DENGUE VIRUS DIVERSITY REVEAL ASSOCIATIONS WITH VIRAL PHYLOGENETIC CLADE AND INTER-HOST DIVERSITY

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Analogous to observations in RNA viruses such as Human Immunodeficiency Virus, genetic variation associated with intra-host dengue virus (DENV) populations has been postulated to influence viral fitness and disease pathogenesis. Previous attempts to investigate intra-host genetic variation in DENV characterized only a handful of viral genes or limited numbers of full-length genomes. We developed a whole-genome amplification approach coupled with deep sequencing to capture intra-host diversity across the entire coding region of DENV-2. Using this approach, we sequenced DENV-2 genomes from the serum of 22 Nicaraguan individuals with secondary infection and captured ~75% of the DENV genome in each sample (range 40-98%). We identified and quantified variants using a highly sensitive and specific method, and determined that the extent of diversity was considerably lower than previous estimates. Significant differences in intra-host diversity were detected between genes and also between immunogenically-distinct domains of the Envelope gene. Interestingly, a strong association was discerned between the extent of intra-host diversity in a handful of genes and viral clade identity. Additionally, the abundance of viral variants within a host, as well as the impact of viral mutations on amino acid encoding and predicted protein function, determined whether intra-host variants were observed at the inter-host level in circulating Nicaraguan DENV-2 populations, strongly suggestive of purifying selection across transmission events. Our data illustrate the value of high-coverage genome-wide analysis of intra-host diversity for high-resolution mapping of the relationship between intra-host diversity and clinical, epidemiological and virological parameters of viral infection.

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DAILY HANDHELD ULTRASONOGRAPHY PERFORMED BY CLINICIANS CAN DETECT SUBCLINICAL PLASMA LEAKAGE AND IDENTIFY DENGUE PATIENTS AT RISK FOR DENGUE SHOCK SYNDROME

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Plasma leakage is the critical feature of severe dengue infection. Timely detection of plasma leakage is important to identify those at risk for dengue shock syndrome (DSS). While presently available clinical and laboratory (e.g. hematocrit) markers have only low sensitivity , ultrasonography may improve timely detection of plasma leakage. Unfortunately, limited availability in resource poor settings and costs are hurdles for widespread implementation. In recent years, however, affordable handheld ultrasound devices have become available that can be operated by non-radiologists. We studied the possibility to detect plasma leakage and to identify patients at risk for DSS by non-radiologists using serial handheld ultrasonography in a prospective cohort of Indonesian patients with laboratory proven dengue. A total number of 66 patients were enrolled, of whom 44 were classified as non-DSS and 11 as DSS. At enrollment, subclinical plasma leakage in the form of ascites or pleural effusion was already detected in 26% of the patients. Presence of ascites or pleural effusion at enrollment had a positive predictive value of 35% for the development of DSS, and a negative predictive value of 90%. At enrollment, 55% of DSS cases already had detectable plasma leakage and this increased to 91% during the subsequent days. Gallbladder wall edema was most pronounced in DSS patients and often preceded ascites and/or pleural effusion. The findings of handheld ultrasonography corresponded well with conventional ultrasonography made by a certified radiologist during the critical phase of the infection. Serial hematocrit and albumin measurements, as recommended by WHO guidelines, failed to identify plasma leakage. In conclusion, serial handheld ultrasonography performed by clinicians is a sensitive tool to detect plasma leakage in dengue, in contrast to existing markers such as albumin and hematocrit. Detection of subclinical plasma leakage and/or an edematous gallbladder wall can identify patients at risk for DSS, and these patients merit more intensive monitoring of circulatory status and intravenous treatment. The introduction of more affordable handheld ultrasound devices, operated by shortly trained clinicians, can increase the clinical implementation of ultrasonography in dengue, especially in resource poor countries which are mostly affected by this devastating disease.

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ELQ-300 FOR TREATMENT AND PREVENTION OF MALARIA

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ELQ-300 is a novel antirespiratory compound with low nanomolar IC_{50} values against blood stages of *Plasmodium falciparum* and *P. vivax*, including drug resistant laboratory strains and field isolates. It has, so far, proven impossible to generate ELQ-300 resistant mutants using single step methodology, with a demonstrated resistance frequency far improved over atovaquone. The *in vitro* potency, combined with the high metabolic stability, results in spectacular oral efficacy with a curative blood stage dose of 1 mg/kg against *P. berghei* or *P. yoelii* infected mice in standard 3 and 4 day tests. Interestingly, the molecule is also exquisitely potent against exo-erythrocytic stages and impacts not only liver schizonts, but also blocks stage V gametocyte development and inhibits ookinete formation in the mosquito midgut. Although its aqueous solubility is limited, ELQ-300 exhibits high oral bioavailability at therapeutically relevant doses with extended half-lives in rodents and dogs. Impressively, *in vivo* doses of 0.03mg/kg result in formal causal prophylaxis and killing of all *P. berghei* liver schizonts; furthermore this same dose results in complete inhibition of *P. berghei* oocyst formation in a mouse feeding study thus totally inhibiting sporogony and demonstrating a 100% block of transmission. ELQ-300 has high *in vitro* selectivity over human cytochrome bc1 and it is without cytotoxicity (10μM) against a panel of mammalian cell types. It was not inhibitory against a large safety and selectivity target panel of receptors, amine transporters and ion channels, including the hERG channel, nor is the compound genotoxic as assessed in a 5-strain Ames and *in vitro* micronucleus assays. Given the low predicted dose in patients, a long predicted human half-life, and potent activity against blood and exo-erythrocytic stages of parasite development, ELQ-300 offers the hope of a new molecule for the treatment, prevention and, ultimately, eradication of malaria.

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POTENTIAL EFFICACY OF CITICOLINE AS ADJUNCT THERAPY FOR CEREBRAL MALARIA

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During cerebral malaria (CM), sequestration of parasitised erythrocytes, platelets and leucocytes within the cerebral microvessels, coupled with cytokine overproduction leads and/or blood brain barrier (BBB) disruption and/or intercellular junction opening. We evaluated the efficacy of citicoline (CTC), a membrane stabilising agent already registered for use in ischemic stroke and anti-histamine as adjunct therapy to enhance the recovery from CM. Initial trials in *Plasmodium berghei* ANKA-infected mice (which develop a lethal syndrome 7 days post-infection) showed that treatment with CTC alone (1g/kg, from day-4 to day-7) doesn't enhanced survival after day-7. After day-14, CTC in combination with a sub-effective dose of artemisinin (40 mg/kg) enhanced survival from 20% (artemisinin alone) to 75% (art+CTC). CTC is a very well tolerate compound, registered by the FDA as a nutritional supplement for children. It could be thus very easy to use in public health structures. These data support development of studies in human to address interest of membrane protector as adjunct therapy during Malaria.

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PATHWAYS TO HEMOLYTIC TOXICITY OF PRIMAQUINE: EVALUATION OF POTENTIAL HEMOTOXIC METABOLITES OF PRIMAQUINE AND AMINOPHENOL ANALOGS *IN VITRO* ON NORMAL AND GLUCOSE 6-PHOSPHATE DEHYDROGENASE DEFICIENT HUMAN ERYTHROCYTES

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Recent malaria treatment measures have resulted into significant decrease in cases of *Plasmodium falciparum*, but *P. vivax* cases are steadily rising. Primaquine (PQ) is the drug of choice for radical cure of relapsing *P. vivax* malaria. However, utility of PQ has been limited primarily due to hemolytic toxicity in the G6PD deficient populations. The redox active metabolites generated through CYP mediated pathways are responsible for hemolytic toxicity of PQ, but the identity of these metabolites has still remained elusive. The phenolic metabolites reported in *in vitro* metabolism and experimental animal studies have been suggested as the potential hemotoxic metabolites. In view of these, 5-hydroxy primaquine (5-HPQ), 8-N hydroxyl 6-methoxyaminoquinoline (NHMAQ) and some aminophenol analogs were evaluated *in vitro* on normal and G6PD deficient human erythrocytes. Hemolytic response was monitored with multiple biochemical markers namely, methemoglobin, reactive oxygen species (ROS) and depletion of reduced glutathione (GSH). The PQ metabolites and aminophenol analogs produced differential hemotoxic response on normal and G6PD deficient human erythrocytes. 5-HPQ and NHMAQ produced a robust increase in methemoglobin and ROS, and the responses were similar in normal and G6PD deficient erythrocytes. However, the metabolites depleted GSH only in G6PD deficient erythrocytes. This differential response may explain selective susceptibility of G6PD deficient individuals to hemolysis during treatment with PQ. 5HPQ produced about 3 fold higher methemoglobin accumulation and more prominent depletion of GSH than NHMAQ. However, NHMAQ generated about three-fold higher ROS signal compared to 5-HPQ. The 2-aminophenols generated more prominent hemotoxic responses than 4-aminophenols, while 3-aminophenols were non-toxic. 4-Methyl and chloro substitutions potentiated the toxicity, while 4- and 5-nitro substitutions attenuated the toxicity of 2-aminophenols. A pattern of structure toxicity relation observed in hemotoxic response of aminophenols may be useful for designing PQ analogs with better therapeutic index.

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AN *IN VIVO* GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PD)-DEFICIENT MOUSE MODEL TO PREDICT HEMOLYTIC TOXICITY OF CANDIDATE 8-AMINOQUINOLINE (8-AQ) ANTI-MALARIAL DRUGS

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Many of 8-aminoquinoline (8-AQ) compounds investigated to treat relapsing malaria are hemolytic in subjects with glucose-6-phosphate dehydrogenase deficiency (G6PDD). As 8-AQ drugs are the only treatment for relapsing *Plasmodium vivax* malaria, research studies to develop non-hemolytic 8-AQs are very important, and an *in vivo* model is desperately needed to test the hemolytic potential of newly developed 8-AQ drugs. We have "proof of concept" for a G6PD-deficient (G6PDD) mouse model with a similar degree of G6PD deficiency as in the human African type

A$^-$ population (10-15% of normal G6PD activity); this model mimics the 8-AQ drug-induced hemolysis in human G6PDD individual. This mouse model was validated using two known hemolytic 8-AQs, *i.e.*, primaquine and pamaquine, and two known non-hemolytic drugs, chloroquine and mefloquine. Major hemolytic parameters evaluated were decreased red blood cell counts; increased reticulocyte counts; Heinz body formation; and decreased haptoglobin level in serum. Mice given the hemolytic drugs consistently displayed a hemolytic response, whereas those treated with chloroquine and mefloquine showed no significant hemolytic response. In this study, we assessed the effects of various dose levels of tafenoquine using this G6PDD mouse model. Tafenoquine was given orally at several doses (13.3, 7.5 or 2.5 mg/kg/d) for 3 days; or (40, 30, 20, or 10 mg/kg/day) for 1 day. A known hemolytic drug, primaquine, was dosed at 8.8 mg/kg/day for 3 days, as the positive control. Tafenoquine at the 100% causal prophylaxis efficacy dose (CP-ED_{100}, 2.5 mg/kg/day for 3 days, or 10 mg/kg for one day) demonstrated no hemolytic toxicity in our G6PDD mouse model, whereas primaquine at ½ ED_{100} (8.8 mg/kg/day for 3 days) displayed a hemolytic response . Higher doses of tafenoquine, above the ED_{100} (e.g. 13.3 mg/kg/d for 3 days, or 40 mg/kg for 1 day), were shown to induc hemolysis in G6PDD mice. We conclude that tafenoquine treatment at the ED_{100} dose is safer than treatment with PQ at the ED_{100} dose.

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A PHASE I STUDY TO INVESTIGATE THE HAEMOLYTIC POTENTIAL OF TAFENOQUINE IN HEALTHY SUBJECTS WITH GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY (TAF110027)

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Tafenoquine (TQ) is an 8-aminoquinoline (8-AQ) with a half life of 2-3 weeks currently in development as a single dose treatment for the radical cure of *Plasmodium vivax* malaria. 8-AQs cause haemolysis in glucose-6-phosphate dehydrogenase (G6PD) deficient individuals; common in malarious regions. As a first step to investigate TQ's haemolytic potential quantitatively we have commenced a dose escalation Phase I safety study. We recruited healthy female volunteers (Hb >12 g/dL) who were heterozygous for the G6PD Mahidol mutation assessed by PCR-RFLP with red cell G6PD enzyme activity 40-60% of normal (based upon testing n=39 males defining a local median value of 11.5 IU/gHb, as reported previously. G6PD normal control subjects were Mahidol negative with >80% G6PD activity. *A priori* we defined a dose escalation strategy based upon the number of subjects reaching a dose limiting toxicity (DLT) defined as an absolute decline of ≥2.5 g/dL Hb or ≥7.5% in haematocrit: should 3 or more subjects reach DLT in any given cohort dose escalation will stop. We have completed dosing cohorts of 6 normal and 6 deficient subjects with single 100 mg and 200 mg doses. 1/6 of the heterozygote subjects in the 100 mg cohort, and 2/6 in the 200 mg cohort reached DLT. Median maximum fall in Hb in both cohorts was 1.6 g/dL (range 0.9 - 2.4 in the 100 mg cohort, range 1.3 g/dL - 3.1 g/dL in the 200 mg cohort). An increase in bilirubin was common (5/6 in 100 mg cohort, 3/6 in 200 mg cohort) and by day 12 all subjects had exhibited a reticulocyte level of at least 2.2%. In G6PD normal subjects smaller Hb falls were observed (range 0.6 to 2.1 g/dL) but were not attributed to hemolysis. Additional relevant clinical and laboratory data will be presented. TQ is a promising new therapy for radical cure of *P. vivax* in G6PD normal subjects. Our data demonstrate that in non-anaemic G6PD deficient heterozygotes with 40-60% G6PD enzyme activity TQ regularly causes haemolytic anaemia, which appears to be dose-related, and has been of mild to moderate degree with a dose of 100-200 mg.

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USING HUMAN BLOOD STAGE *PLASMODIUM FALCIPARUM* INFECTION TO DEFINE THE ACTIVITY OF LICENSED AND EXPERIMENTAL ANTIMALARIAL DRUGS

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The growing awareness of slow clearance as a potential marker of incipient failure of artemisinin antimalarials has highlighted the need for better defining the clearance kinetics of parasitemia following drug therapy. Most previous studies have used parasite counts as determined by slide estimation to determine clearance. Such data are of suboptimal quality to precisely define the inherent activity of various antimalarials. We have undertaken a series of four studies in human volunteers experimentally infected with blood stage parasites of *Plasmodium falciparum* where the clearance kinetics of parasitemia have been closely monitored by serial quantitative PCR. Data will be presented on the kinetics of clearance, presented as the parasite reduction ration (PRR) in these four cohorts following treatment with artemether-lumefantrine, atovaquone-proguanil, pyrimethamine-sulfadoxine and mefloquine. Benchmarking the pharmacodynamic activity of both licensed and experimental antimalarials and correlating these data with the pharmacokinetic profile of the drugs will provide a much needed evidence base for the development of new antimalarials as well as improving understanding drug activity and the development of drug resistance.

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A POPULATION PHARMACOKINETICS ANALYSIS OF OZ439 DISPOSITION IN PATIENTS

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OZ439 is a synthetic trioxolane undergoing clinical investigation and being developed by MMV as a potential anti-malarial therapy. OZ439 has shown *in vivo* a potential for a single dose cure in *Plasmodium falciparum* malaria while showing in *in vitro* and *in vivo* models marked prophylactic potential, extended half-life and oral bioavailability, and increased metabolic stability. Data from Phase I study in healthy volunteers and the on-going Phase IIa have confirmed OZ439 to be safe and tolerable. The pharmacokinetics (PK) of OZ439 will help determine its potential as a single dose cure. PK data from the on-going Phase IIa investigation in patients was analysed using a non-linear mixed effects approach. In this study single doses of OZ439 have been orally administered to patients. The optimal structural model was a 2 compartment model with oral absorption. The oral absorption had a lag time associated with it. Inter-individual variability was attributed to the central volume of distribution, clearance and lag time. The observed PK was linear with a clearance of 65.7 L/hr and an elimination half-life of 53 hours; thus the PK supported OZ439's potential as a single dose cure. This population PK model will aid in predicting the time course of OZ439 at different doses and will be updated as more data is collected. This will include determining the covariates of the PK parameters in order to characterise the effects of age, co-administered drugs, and ethnopharmacology. A major benefit of characterising the PK in this manner is the ability to use these parameters

in PK/PD models, to make population predictions of OZ439's efficacy as an anti-malarial, to confirm the suitability of OZ439's PK for a single dose cure and to design future clinical trials.

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A CONSERVED *PLASMODIUM* PROTEIN REGULATES MEROZOITE PRODUCTION DURING INTRAERYTHROCYTIC SCHIZOGONY

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A critical aspect of *Plasmodium* biology is its ability to multiply exponentially during different stages of its life cycle. Both within the vertebrate and invertebrate hosts, the parasite undergoes a rapid increase in population through asexual reproduction, which is critical for its transmission and disease pathogenesis. In the human blood stages, *P. falciparum* typically produces an average 20 merozoites per schizont, which upon release, infect new host erythrocytes and continue to exponentially increase in numbers. Although this is a crucial step in maintaining infection in the human host, mechanisms controlling merozoite production are still poorly understood in *Plasmodium*. Here, we report the identification of a conserved *Plasmodium* protein, critical for regulating merozoite production in the intraerythrocytic stages of *P. falciparum*. piggyBac insertion into the coding sequence of this protein results in approximately 40% reduction in merozoite numbers and severely attenuates the parasite intraerythrocytic growth rate. Understanding the functions of this protein will provide novel insights into a crucial component of parasite biology.

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GENOME-EDITING THE MALARIA PARASITE *PLASMODIUM FALCIPARUM*

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Malaria afflicts 225 million people worldwide and its most lethal etiologic agent, *Plasmodium falciparum*, is evolving to resist the latest-generation therapeutics. Tools for genome-directed study of such resistance and ways to overcome it, however, are sorely lacking. Here we report rapid and targeted genetic engineering of this parasite, using zinc-finger nucleases that produce double-strand breaks in a user-specified locus and trigger homology-directed repair. Targeting an integrated *gfp* locus, we obtained a homogeneous population of knockout parasites in an unprecedented 15 days. Moreover, ZFNs engineered against *pfcrt* produced parasites that carry a panel of investigator-defined point mutations and acquired antimalarial drug resistance. The efficiency, versatility and precision of this approach enable genome editing of this human pathogen to meet the challenge of substantially reducing the burden of disease.

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GLOBAL IDENTIFICATION OF PALMITOYLATION SITES IN *PLASMODIUM FALCIPARUM* SCHIZONTS

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Protein palmitoylation, the addition of a 16-carbon saturated fatty acid to cysteine residues, is a major post-translation modification that is used in all eukaryotes to regulate protein function. Like phosphorylation, palmitoylation is a reversible event, and can serve to regulate protein localisation, stability, and activity either stably or in response to specific stimuli. Previously only three *Plasmodium falciparum* proteins were known to be palmitoylated, but by combining two established and orthogonal purification approaches we have recently identified more than 300 palmitoylated proteins in late blood-stage *P. falciparum* parasites, including palmitoyl-proteins involved in protein secretion, drug resistance, signalling, development, cytoadherence and invasion. This suggests that palmitoylation is likely to regulate multiple *Plasmodium*-specific biological processes, but in order to drive specific biological hypotheses it is critical that we identify which specific sites within each palmitoyl-protein are modified. Like phosphorylation, palmitoylation sites can largely not be predicted using primary sequence data alone, with the exception of cysteines immediately downstream of myristoylation sites. We have now developed a novel protocol that can identify specific palmitoylation sites, and have used it to identify more than 1000 palmitoylation sites in *P. falciparum* blood stage proteins, some of which are now being followed in functional studies. Given the scale at which this modification is used in blood stage parasites, palmitoylation should be considered alongside phosphorylation as a major regulatory mechanism and potential target for intervention.

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DISCOVERY OF CONSERVED *PLASMODIUM* ANTIGENS ON THE SURFACE OF RED BLOOD CELLS USING DNA APTAMERS

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During its intraerythrocytic cycle, the human malaria parasite *Plasmodium falciparum* remodels the host red blood cell (RBC) plasma membrane with a poorly defined collection of antigenically diverse proteins. While these proteins play roles important for parasite survival and virulence they are exposed to potential drugs and immune effectors in blood for ~24 hours as parasites develop. It is not clear, however, whether any of these proteins are structurally conserved enough to provide a novel target for vaccine or drug development. Here we describe a novel DNA aptamer selection scheme to probe for epitopes on the parasitized RBC surface that are conserved between geographically distinct parasite isolates. Our scheme isolated 14 aptamers that tightly bind parasitized RBCs (with low-nanomolar dissociation constants) and possess no affinity for non-parasitized RBCs. Three aptamers recognize all laboratory-adapted clones and field isolates of *P. falciparum*, *P. vivax* and *P. knowlesi* tested yet show no affinity for the murine malaria parasites, *P. berghei* and *P. chabaudi*. Moreover, some of these aptamers efficiently kill blood-stage parasites *in vitro* in a dose-dependent and sequence-specific manner. Current work is focused on identifying the aptamer binding to membrane extract targets and elucidating their role in parasite survival. Discovery of a protein or epitope conserved between the major species of human malaria parasites may have implications for drug and vaccine development and validates our aptamer selection scheme as a powerful tool for antigen discovery.

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TOWARD RNA-BASED APPROACHES FOR STUDYING MALARIA PARASITE BIOLOGY

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Malaria caused an estimated 655,000 deaths in 2010, mostly in children under the age of five years. *Plasmodium falciparum* is responsible for the majority of malaria morbidity and mortality. Research efforts aimed at gaining biological insights into this parasite are hampered by a lack of gene regulatory technologies that permit elucidation of genetic pathways in situ. Inducible expression is an important strategy for establishing how genes function, and for determining their interactions in biological pathways. Pathways that are both essential and unique to the parasite can then be targeted in drug development efforts. Based on this need, we have reconstituted an inducible T7 RNA polymerase (T7 RNAP) transcriptional system in *P. falciparum*. We have demonstrated T7 RNAP-directed, IPTG-inducible target transcript production in situ at levels similar to or greater than those generated by characterized native polymerase II promoters. In the repressed state, target transcript levels are reduced by more than ten-fold. With this new tool, we envision using RNA-based approaches, such as conditionally expressing aptamers that can disrupt protein function, to complement the characterization and validation of potential parasite drug targets.

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BASELINE AND EARLY POST-ANTIPARASITIC TREATMENT URINE ANTIGEN LEVELS IN NEUROCYSTICERCOSIS

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Circulating antigen levels may provide a monitoring tool for human neurocysticercosis (NCC). Experience with serum levels shows that they correlate with parasite burden and evolution, and that they drop in a few months after successful antiparasitic treatment. Also, under clinical circumstances, urine levels have been shown to correlate with serum levels. Urine being a non-invasive sample, we wanted to evaluate whether it could be used as a follow up tool to determine early changes in levels of circulating antigen, during the initial two weeks of therapy. Thirty-one patients, 18 men and 13 women, aged 18 to 80 years, with neurocysticercosis demonstrated by MRI and confirmed by antibody serology on EITB, had urine samples collected at baseline and at days 1, 3, 5, 7, 9 11, and 13. These samples were processed using a monoclonal antibody-based AgELISA (B158/B60). Sensitivity of the AgELISA was 81% (25/31) both in serum and in urine. There was a very high correlation between baseline serum and urine levels. Antigen levels did not become negative in this short period and had different trends, mostly stable, or decreasing. In some patients, antigen levels decrease by more than 10 times along two weeks. Patients who were later shown as treatment failures had persistent or increasing antigen levels. Urine antigen detection may serve to monitor NCC patients after antiparasitic treatment, particularly in those who are antigen positive at baseline.

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IMMUNE RESPONSE DURING NEUROCYSTICERCOSIS IN MADAGASCAR

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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-*Taenia solium* results. We thus urgently need to improve diagnostic of NCC. We analysed cellular and serological immune response in adult patients suffering from recent seizures, with or without images of NCC on CT-Scan. We used both reference glycosylated proteins and liquid of cysts (LC) as antigen for lymphocyte proliferation tests (LPT) and for serological analysis. In the same time we compared anti-Tsolium isotypes (IgE, IgA, IgD, IgG) in blood and in cerebrospinal fluid (CSF). LPT using LC but not glycosylated protein was found to be an accurate method to detect cysticercosis which pave the was to the development of new strategy of test for this disease. In the same time isotype analysis enlightened clearly local secretion of antibodies in CSF and IgD was more accurate to detect this proliferation than IgA or IgE. An overall analysis of these data will be presented.

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ENHANCED CORTICOSTEROID USE IN THE TREATMENT OF PARENCHYMAL NEUROCYSTICERCOSIS REDUCES SEIZURE OCCURRENCES WITHOUT A REDUCTION IN EFFICACY OR AN INCREASE IN SIDE EFFECTS

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Although corticosteroids are commonly employed to reduce treatment induced seizures in the treatment of parenchymal neurocysticercosis (NCC), the benefits of their use and exactly how and when to use them has not been rigorously studied. In an open randomized trial, two groups of 31 patients with one or more viable parenchymal cysts were treated with 15 mg/kg albendazole for 14 days and either the standard dexamethasone regimen at 6 mg/day for 10 days, (Arm 1) or for 28 days at 8 mg/day with a 14 day taper (Arm 2). Subjects were required to be on antiseizure medication with proven effective blood levels. The number of seizures and persons with seizures were compared from 1-360 days with the interval of 11-42 days as the primary outcome, efficacy at 180 days by MRI and side effects from 1-360 days. Number of seizures (mostly partial seizures) and people with seizures were increased in Arm 1 compared to Arm 2 during the trial but did not reach significance for the primary outcome 11-42 day interval (p=0.623). There was a similar increase of generalized seizures in Arm1 but too few occurred in either Arm to be analyzable. However, from days 1-10 and 11-21 the number of partial seizures (p=0.016 and p=0.016 respectively) and people with seizures (p=0.013 and p=0.020 respectively) were significantly increased in Arm 1 compared to Arm 2. There was also a significant increase of partial

seizures (p=0.036) and persons with seizures (p=0.041) in Arm 1 during the entire course of the trial, mostly due to early events. By a number of measures efficacy including percent reduction in cysts cure rates, and subjects requiring retreatment, was similar between the Arms. Although there were more adverse events in Arm 1, mostly due to neurologically based changes, none of the events reached significance. Enhanced corticosteroids significantly decreased treatment induced seizures compared to a standard regimen without a decrease in efficacy or undo increase in side effects. Effects were due predominately to a decrease in seizures within the first 21 days.

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TWO LARGE EPILEPSY SURVEYS IN A CYSTICERCOSIS-ENDEMIC REGION IN TUMBES, PERU

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Epilepsy is one of the older diseases of mankind. The prevalence of epilepsy worldwide added to inadequate treatment and late intervention results in chronic morbidity and considerable mortality in poor populations. Neurocysticercosis (NCC), a helminthic disease of the central nervous system, is one of the leading causes of seizures and epilepsy. Taking advantage of a large cysticercosis elimination program, we performed two wide scale cross-sectional studies in 58 rural communities of the Northern coast of Peru to assess the prevalence and characteristics of epilepsy and epileptic seizures in this cysticercosis-endemic region. Two studies were conducted between 2006 and 2007, involving 20,610 individuals. An initial epilepsy screening survey was followed by a phase of medical evaluation, followed by interview with a neurology specialist. A total of 17,452 individuals older than 2 years (86.41%) consented to participate. The overall prevalence of epilepsy was 17.25/1000, and that of active epilepsy was 10.8/1000 inhabitants, without marked differences between surveys.The prevalence of epilepsy by age increased after age 25 years and dropped after age 45. Only 45 out of 188 (23.94 %) patients with active epilepsy (30/107 and 15/81 from 2006 and 2007 respectively) were taking antiepileptic drugs. All of them were receiving sub-therapeutic doses. The seroprevalence of antibody against T. solium in individuals with epilepsy was approximately 40% in both studies. In the first survey there was no statistically significant difference in overall seroprevalence between individuals with and without epilepsy. The proportion presenting strong antibody reactions (4-7 bands by EITB) was however five times higher in individuals with epilepsy than that in individuals without epilepsy. In the second survey, the seroprevalence as well as the proportion presenting strong antibody reactions were significantly higher in individuals with epilepsy. Brain CT showed NCC-compatible images in 109/282 individuals with epilepsy (39%). All individuals with viable parasites on CT were seropositive. Prevalence of epilepsy in this cysticercosis endemic region is high and NCC is an important contributor to it.

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PREVALENCE OF EPILEPSY IN 60 VILLAGES OF THREE PROVINCES OF BURKINA FASO

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Taenia solium cysticercosis is considered as an emerging infection in Sub-Saharan Africa. Results from a previous pilot study in Burkina Faso showed that half of people with epilepsy in two villages where pigs were raised had neurocysticercosis. The aim of this study was to estimate the lifetime prevalence of epilepsy in 60 villages located in three Provinces of Burkina Faso. This is the baseline cross-sectional component of a large community randomized-control trial which took place between February 2011 and January 2012. The provinces of Nayala (10 villages), Boulkiemdé (30 villages) and Sanguié (20 villages) were selected for inclusion in the study. In each province, all departments where pigs were raised (30 of 31) were selected. Within each department, two villages meeting the eligibility criteria were selected at random. In each village, one person was selected at random from each of 10 (with sows), 30 (with piglets) and 40 (with or without pigs) randomly-selected concessions (a grouping of several households). A total of 60 of 80 participants in each village were asked for their consent to provide a blood sample to test for the presence of antigens to the larval stages of *T. solium* using an ELISA test. A total of 4,970 individuals, aged 6 to 99 years (median of 30 years), were interviewed with a screening questionnaire for epilepsy. Screened positive participants were examined by a physician to confirm the diagnosis of epilepsy. Preliminary estimates of the unweighted lifetime prevalence of confirmed epilepsy were 3.0%, 1.0% and 3.4% in Boulkiemdé, Nayala and Sanguié, respectively. At the village-level, the lifetime prevalence showed important variation, ranging from 0% (in 7 villages, including 4 villages in Nayala Province) to 8.8% in one village of Sanguié. Although these data are being analysed to account for the clustered nature of the sampling strategy, they provide evidence that the prevalence of epilepsy varies spatially in rural Burkina Faso.

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CYST STAGE AND DIMENSION INFLUENCE SEROLOGICAL RESPONSE IN HEPATIC *ECHINOCOCCUS GRANULOSUS* INFECTION

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Diagnosis of hepatic cystic echinococcosis (CE) is based primarily on ultrasound (US) and serology. The latter has a minor role as it cannot distinguish between active and inactive cysts and its sensitivity and specificity are poor and influenced by many variables. Furthermore, most clinical studies on serology of CE do not take into account the cyst stage and this impairs their usefulness in clinical decision making. We investigated retroactively a cohort of patients diagnosed with CE who were seen in our clinic from May 2000-June 2012, by looking at the correlation, if any, of their cyst stage as seen on US and their serological test (IHA and ELISA) results. Cysts were stratified by stages (active, transitional and inactive cysts, WHO IWGE) and by dimension (S≤ 5 cm, M 5-10 cm and L≥ 10 cm). Of the 339 patients evaluated in 812 visits, 249 had 1 parasitic cyst and 90 had a non parasitic cyst. IHA and ELISA were positive in 87 and 83% of active cysts, 90 and 93% of transitional and 60 and 50% of inactive cysts and post-surgical residual cavities, respectively.

Specificity of both tests was 99 %. A statistically significant difference (p< 0,0001) for median values of IHA and ELISA was found between active and inactive cysts, active and residual cavities and transitional and inactive cysts. Both tests were positive in 58% of examinations, specifically in 81% of active cysts, 90% of transitional cysts and 51% of inactive cysts. Both tests were negative in 99% of non parasitic cysts. Serological response is also influenced by cyst dimensions. Both tests were positive in 76% of L , 62 and 48% of M and S cysts respectively, with statistically significant differences between serological response among L and S cysts and M and S cysts. Serological response to hepatic CE with routinely used tests is influenced by dimension and biological activity of the cyst. Future clinical studies on the use of serology in CE should take this into account.

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TH17/TREG IMBALANCE IN PATIENTS WITH LIVER CYSTIC ECHINOCOCCOSIS

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Echinococcosis is a chronic parasitic infectious disease regulated by T cell subsets. CD4+CD25+FoxP3+ regulatory T (Treg) cells and Th17 cells have been described as two distinct subsets and have the opposite effect on inflammation. Th17/Treg balance controls inflammation and may play important role in the pathogenesis of immune evasion. To assess whether this balance was broken, we detected Th17/Treg functions in different levels including cell frequencies, related cytokines secretion and key transcription factors in patients with cystic echincoccosis and healthy controls. The results demonstrated that patients with cystic ehinococcosis revealed significant increase in peripheral Treg number, related cytokines (IL-10 and TGF-β1) and transcripition factor (Foxp3) levels and moderate decrease in Th17 number, related cytokines (IL-17 and IL-23) and transcription factor (RORγt) levels as compared with controls. Results indicated that Th17/Treg functional imbalance exsists in patients with choronic cystic ehinococcosis, sugessting a potential role for Th17/Treg imbalance in the pathogenesis of immunue evasion in echinococcosis.

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USE OF SMARTPHONES IN HEALTH AND DEMOGRAPHIC SURVEILLANCE

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Health and Demographic surveillance systems (HDSS) can provide essential information in areas where routine vital registration is absent or incomplete. HDSSs also play an essential role in health intervention studies in such areas. Setting up and running an HDSS poses an operational challenge, and a reliable and efficient platform for data collection and management is a key prerequisite. openHDS is an HDSS data system that provides data entry, quality control, and reporting to support demographic and health surveillance. openHDS has recently been integrated with the Open Data Kit (ODK) software platform for data collection using mobile devices running the Android operating system. The use of direct data entry using smartphones offers a number of advantages: it reduces the workload of the data management team, allows for near real-time quality control, and can provide guidance for the project logistics. Here we present an overview of the openHDS/ODK software platform, and report on the experience of using this platform to set up a HDSS to support a malaria intervention study in Kenya, in the Rusinga Island during the Solarmal Project in collaboration with the ICIPE research center in Mbita, Nyanza (Kenya).

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ROLE OF POOR ENVIRONMENT AND SOCIAL STATUS ON *LEPTOSPIRA* TRANSMISSION IN THE URBAN SLUM SETTING: A PROSPECTIVE PROPENSITY SCORE-MATCHED COHORT STUDY

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Leptospirosis is an important health problem in slum settlements worldwide, which have environmental and social conditions for rat-borne transmission. We performed a propensity score-matched analysis of longitudinal data from a cohort of urban slum residents to delineate the role of poor environment and social status on Leptospira infection risk. A prospective study identified Leptospira infections, potential environmental transmission sources, socioeconomic factors, and risk behaviors among slum residents during four annual serosurveys. We calculated a propensity score to estimate the probability of an individual residing in proximity to environmental transmission sources, based on 13 socioeconomic variables, and a second score to estimate the probability of having a per capita daily household income below the median of $1.32, based on 16 environmental variables. We then created two matched cohorts based on these propensity scores in order to evaluate the isolated infection risk due to slum environment and social status, respectively, in Generalized Estimation Equation models. Among 1224 pairs of person-years of follow-up, matched according to propensity for environmental exposures, we found the 15-24 year (RR, 2.60, 95% CI, 1.29 - 5.23) and 25-34 year (RR, 3.29; 95% CI, 1.61 - 6.72) age groups, male gender (RR, 2.15; 95% CI,1.31 - 3.52), illiteracy (RR, 2.94; 95% CI, 1.68 - 5.17), and household proximity to open waste sewers and flood risk areas (RR, 1.90; 95% CI, 1.03 - 3.50) to be independent risk factors. Among 1315 pairs of person-years of follow-up, matched according to propensity for low income, we found belonging to 25-34 year age group (RR, 3.13; 95% CI, 1.53 - 6.42), male gender (RR, 2.17; 95% CI, 1.34 - 3.50), illiteracy (RR, 1.91; 95% CI, 3.47 - 1.05), and household proximity to open sewers and flood risk areas (RR, 2.06; 1.16 - 3.66) to be independent risk factors for infection. We found that poor environmental conditions associated with inadequate sewage and rainwater drainage significantly increase the risk of leptospirosis transmission, independent of economic status. Young adult male and illiterate participants were also at greater risk. Prevention of urban leptospirosis will therefore require improving infrastructure in slum communities. Furthermore, the specific exposures and risk behaviors of young males and individuals with low social status need to be identified in order to mount effective interventions.

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LESSONS LEARNED IN IMPLEMENTING LOW-COST EHEALTH TOOLS IN NICARAGUA: SUPPORTING INFORMATION COLLECTION, MANAGEMENT AND USE IN HEALTH CARE DELIVERY AND PUBLIC HEALTH RESEARCH IN LIMITED-RESOURCE SETTINGS

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This is a critical time in the global dialogue about "eHealth". Investing in efficient, accessible, and cost-effective information and communication technology (ICT) tools can help to improve health outcomes and prevent diseases in low-resource settings. Since 2004, the Sustainable Sciences Institute has been working with various eHealth and mHealth tools to support clinical and epidemiological data management needs for the Pediatric Dengue Vaccine Initiative cohort study, in collaboration with the University of California, Berkeley, and the Nicaraguan Ministry of Health. Beginning in 2009, several collaborative projects were launched in Nicaragua with local partners to adapt, test and implement various ICT tools to improve timely and efficient access to information for key healthcare actors at the primary care level. Work includes implementation at various scales of a web-based electronic medical record system (OpenMRS) for pediatric immunization tracking and prenatal health monitoring and follow-up. In 2010, an open-source web-based Laboratory Information Management System (LIMS) was developed for the National Diagnostic and Reference Laboratory and its regional centers, which is currently in the implementation phase. In 2011, a primary care blood transfusion recipient tracking system was developed, linking the Red Cross blood donation information system with that of the national blood bank commission. In parallel, work with OpenROSA-compliant open-source mobile health technologies including OpenXData, OpenDataKit, CommCare, EpiSurveyor, and FrontlineSMS is ongoing and aims to extend the reach of data collection and reporting tools at both the clinic and community levels. These applications include support of the Behavior Change Communication project working with men in rural areas, pregnancy and child emergency notification systems, rapid notification of communicable diseases, and decision support for community surveillance, all using phones as primary data collection instruments. Critical lessons learned include engaging primary stakeholders early and often in the iterative processes of design, implementation and evaluation of these interventions. This helps to ensure that changes in work flow and information flow facilitated by ICTs are incorporated in a sustainable way to support health system strengthening.

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MORTALITY TRENDS FROM 2003 TO 2009 AMONG ADOLESCENTS AND YOUNG ADULTS IN RURAL WESTERN KENYA USING A HEALTH AND DEMOGRAPHIC SURVEILLANCE SYSTEM

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Targeted global efforts to improve survival of young adults need information on mortality trends; contributions from health and demographic surveillance system (HDSS) are required. Retrospective analysis of deaths among adolescents (15-19 years) and young adults (20-24 years) was conducted using census and verbal autopsy data in rural western Kenya under HDSS. Mid-year population estimates were used to generate all-cause mortality rates per 100,000 population by age and gender, by communicable (CD) and non-communicable (NCD) causes. Linear trends from 2003 to 2009 were examined. In 2003, all-cause mortality rates of adolescents and young adults were 403 and 1,613 per 100,000 population among females, and 217 and 716 per 100,000 among males, respectively. CD mortality rates among females and males 15-24 years were 500 and 191 per 100,000 (relative risk [RR] 2.6; 95% confidence intervals [CI] 1.7-4.0; $p<0.001$). NCD mortality rates in same aged females and males were similar (141 and 128 per 100,000, respectively; $p=0.76$). By 2009, young adult female all-cause mortality fell 53% (χ^2 for linear trend 30.4; $p<0.001$) and 61.5% among adolescent females (χ^2 for linear trend 11.9; $p<0.001$). No significant CD mortality reductions occurred among males or for NCD mortality in either gender. By 2009, all-cause, CD, and NCD mortality rates were not significantly different between males and females, and among males, injuries equalled HIV as the top cause of death. Significant reductions in adolescent and young adult female mortality rates evidence the effects of targeted public health programmes, however, all-cause and CD mortality rates among females remain alarmingly high. Data underscore the need to strengthen programmes and target strategies to reach both males and females, and to promote NCD as well as CD initiatives to reduce the mortality burden among both genders.

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MALARIA RAPID DIAGNOSTIC TESTS IN CONTEXT: INSIGHTS FROM THE ACT CONSORTIUM

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The availability of affordable, accurate Rapid Diagnostic Tests (RDTs) for malaria is enabling a shift from presumptive treatment to parasitological confirmation. Questions remain as to where RDTs should be deployed, best practice to support their effective deployment and their potential impact and cost under real-life conditions. Key determinants have been proposed to include the epidemiological setting, prior provider and community experiences and practices and the presence and effectiveness of supporting interventions. This paper will present a framework for considering the complex issues of RDTs as they are implemented in context, focusing on steps along a pathway from initial diagnostic policy choice through uptake and provider "adherence" to test results and on into public health impact and cost-effectiveness. The presentation will draw on the multidisciplinary work of projects within the ACT Consortium from 9 countries in Africa and Asia and present empirical data from these studies and others from the literature. This will include experiences from the introduction of RDTs through public and private sector providers, and at the community level in a variety of epidemiological and health system settings.

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DETECTION OF HUMAN MONKEYPOX IN THE REPUBLIC OF THE CONGO FOLLOWING INTENSIVE COMMUNITY EDUCATION

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In October, 2009, interethnic violence in northwestern Democratic Republic of the Congo (DRC) precipitated the movement of refugees across the Ubangi River into neighboring Republic of the Congo (ROC). By the end of January 2010, approximately 114,000 refugees had relocated to cities and villages along the river in ROC, concentrating mainly in areas north of the city of Impfondo, where medical resources are scarce. Monkeypox is an acute viral infection with a clinical course resembling smallpox. It is endemic in northwestern DRC, but appears to occur only sporadically in ROC. The influx of refugees to ROC heightened concerns about monkeypox in the area, owing to the possibility that virus could be imported, or that incidence could increase due to food insecurity and over reliance on bush meat. As part of a broad-based campaign to improve health standards in refugee settlement areas, UNICEF sponsored a program of intensive community education which included modules on monkeypox recognition and prevention. In April and May, 2010, INCEF, the implementation partner for the program, performed outreach in 25 cities and villages where refugees had congregated. Approximately 65,000 people attended the outreach sessions. In the six months immediately following the outreach, ten suspected cases of monkeypox were reported to health authorities. Skin lesion specimens were collected from 5 of the suspected cases. Laboratory testing confirmed monkeypox virus infection in 2 individuals (one of whom was in a cluster of 4 suspected cases), and one individual was positive for yaws. Analysis of the viral genome of an isolate recovered from 1 of the 2 confirmed cases is highly similar (but not identical) to the virus implicated in a hospital-associated outbreak of monkeypox that occurred in Impfondo, ROC in 2003. It is less similar to a strain isolated from northwest DRC in 2009. Anecdotes collected at the time of case reporting suggest that the outreach campaign contributed to detection of suspected cases by generating a heightened awareness of monkeypox in refugee settlement areas.

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HIGH INCIDENCE OF BURN-RELATED INJURIES IN A DENSELY POPULATED URBAN SLUM IN KENYA

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We examined the household incidence of burn-related injuries using a prospective, population-based infectious disease surveillance system consisting of approximately 28,000 individuals living in 6,000 households in the urban slum of Kibera, in Nairobi, Kenya. The study period was 5 years, July 2006-June 2011. A total of 3,072 cases (2,723 individuals) of burn injury were identified with an incidence of 27.9/1000 person years of observation (PYO). Burn incidence among children <5 years of age was 81.5/1000 PYO compared to 21.2/1000 PYO in those ≥ 5 ($p<0.001$). Females ≥ 5 sustained burn injuries at a rate 1.4-fold greater than males ≥ 5 (24.5 vs. 18.1/1000 PYO; $p<0.001$). The disparity was greatest for women 18-34 and 35-49 years age, who were 1.9-fold ($p<0.001$) and 2.1-fold ($p<0.001$), respectively, more likely to incur a burn injury compared to men of the same age group. Clinical data from a small proportion of all burn cases showed that 82% of burns were due to cooking, the remaining 18% was due to various non-cooking related accidents and other causes (i.e., electrical burns, assault, etc.). Overall burn injury rates from Kibera were 5-fold and 10-fold higher than rates from an urban regional study in Ghana and a national survey in Bangladesh, respectively. Burn injuries may contribute more significantly to increased morbity in the developing world than previously thought and are potentially impacted by urbanization where dense population and unsafe cooking environments may increase the risk.

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THE MARKET IMPACT OF AN INTERNATIONAL COLLABORATION FOR QUALITY CONTROL OF RAPID DIAGNOSTIC TESTS FOR MALARIA

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The number of commercial malaria rapid diagnostic tests (RDT) has expanded over the past decade. The dynamic flux of products, together with weak regulation and a lack of consistent data on quality, has made quality-based procurement difficult. Over several years, WHO, TDR, FIND, US CDC, and other partners developed and operationalized malaria RDT Product Testing (PT) and Lot Testing (LT) programmes. Data collected since 2008 provides unique insight into the impact of such a programme on RDT quality and markets. Through open calls for expression of interest (2008-2011) to ISO13485-certified manufacturers, RDTs were submitted for evaluation against panels of low and high density cultured *P. falciparum* (Pf) parasites, wild-type Pf and *P. vivax* (Pv) parasites, and parasite-negative samples. Similarly, lot testing open to manufacturers and procurers uses the same, though much smaller, sets of Pf, Pv and malaria-negative samples. In 2011, surveys of manufacturer sales (2007-2010) were conducted. To date, three rounds of PT have been performed on 120 products, including 24 resubmissions. The average panel detection score (PDS) against low density samples increased between Rounds 1 and 3, by 9.6% for Pf and 11% for Pv. The average change in PDS at low parasite density for resubmitted products was greater, at 12.7% for Pf and 27.4% for Pv. Based on sales information from 31 manufacturers, the RDT market has increased from 45M in 2008 to 88M in 2010. Sales have shifted towards products with a higher PDS, a surrogate for analytic sensitivity. Lot testing requests have increased by 258%, from 139 in 2008 to 360 in 2011, representing ~50% of lots sold into the public sector; furthermore, LT failure rates have decreased. The malaria RDT evaluation scheme provides data to distinguish between well and poorly performing tests, which in turn informs procurement decisions and enables manufacturers with better tests to expand their markets. The dramatic improvement of resubmitted RDTs indicates that manufacturers can improve product quality when an oversight mechanism is in place.

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VALIDATION OF AN AUTOMATED RDT READER AND DATA MANAGEMENT DEVICE IN TANZANIA

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Lack of proper quality assurance is perceived as a significant obstacle to the widespread implementation of RDT-based malaria management strategy, as recommended by WHO. Previous experience has shown that continuous training and/or job aids can abate reduction in diagnostic accuracy of RDT based diagnosis over time resulting from human error. As well, reporting of diagnostic events is very limited, imprecise and slow in most remote areas, impeding proper decision making by control program managers. Fio Corporation has developed a system to address both problems: improving quality of RDT based diagnosis by providing job aids for RDT processing, automated interpretation through digital technology and optimal case real time reporting using transmission over cell phone network. A fully blinded study was conducted in Bagamoyo district of Tanzania to test the diagnostic accuracy of the Fio system using SD Bioline malaria Pf/Pan RDT. Patient population consisted of males and females > 1 y. o., with symptoms of acute malaria. Main statistical analysis by a third party included dx performance of RDT interpreted by device (DEV), dx performance of RDT interpreted by experts visual (VIS), and comparison of DEV and VIS. Reference standard: expert microscopy performed at a central location. RT-PCR was used as tie-breaker in discrepant results. 1346 patients were enrolled over a 6 week period. Overall Pf infection prevalence was 11.1%. DEV Sens: 95.3; Spec: 94.9; PPV:70.3, NPV:99.4. VIS Sens: 94.7; Spec: 95.6; PPV: 72.8; 99.3. Percentage concordance between DEV and VIS was 97.8. User errors were documented in17/29 (59%) of discrepant results. Data from devices reached the Fio Cloud in real-time and could be accessed by PI & study coordinator. The Sens and Spec obtained are similar to other publications. Fio System was shown to deliver an automated high diagnostic performance, as good as expert visual interpretation. The system was found to be user friendly, practical, reliable and accurate. DEV false positives represented <1% of results.

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EVIDENCE-BASED ANALYSIS OF WHEN TO SWITCH TO A COMBO MALARIA RAPID DIAGNOSTIC TEST IN LIBERIA

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In West Africa *Plasmodium falciparum* (Pf) predominates, accompanied by non-Pf species in mixed infections that can account up to 20% of all malaria cases, with relatively few non-Pf monoinfections. Mixed infections have led some countries to select combo RDTs for routine use. In countries where non-Pf monoinfections account for less than 5% of all malaria cases, pf-only RDTs are preferable as interpretation of tests results is simpler and cost is lower. In 2011 Liberia's NMCP with support from PMI/IMaD proposed monitoring malaria parasite species to respond to claims from providers saying Pf-only RDTs fail to detect malaria because non-Pf monoinfections were on the rise. To contain cost and ensure sample was representative of the population, parasite species identification (ID) was done on biological material collected during MIS 2011, and owned by the NMCP. Species ID based on MMEQA slides -excluding heavy parasitemias- were assumed to introduce selection bias, overestimating non-Pf. During MIS household parasitemia surveys, in addition to mRDTs, slides for MM and blotted blood for PCR were collected. Blind species identification was done at Liberia's National PH Reference Laboratory and IMaD Office, with participation of three expert microscopists (Level 1). MIS 2011 collected blood from 3841 children. A random subsample of 476 slides (15%) were selected for MMQA and preliminary analysis of species ID based on thick blood films (thin films are better for species ID, but were not available). 70 contained malaria parasites. Two slides out of 70 had mixed infections (Pf+Pm) and 13 had only Pm, the rest were Pf exclusively. If these preliminary results are confirmed by the analysis of species in the whole set of positive and readable slides, this will signify that about one fifth of malaria cases have non-Pf monoinfection. Those are not detectable by Pf-only mRDTs, and there will be need -after using up existing stocks- to switch to a combo mRDT with the added cost of retraining, and printing job aids. Decisions such as switching from Pf-only to combo RDTs or from ACT to a different medication is expected to be done on an annual basis or less frequently. A sample size of 750 individuals per stratum (i.e. health region) is easily attainable with biennial parasitemia surveys that reach about 4000 children.

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FACTORS ASSOCIATED WITH ANTIMALARIAL TREATMENT OF MALARIA PARASITE-NEGATIVE PATIENTS AT HEALTH FACILITIES IN THREE REGIONS OF TANZANIA: 2010-2012

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Over-treatment of malaria is a common problem in many malaria-endemic countries, with parasite-negative patients often receiving an artemisinin-based combination therapy (ACT) or other antimalarials, leading to over-use of ACT, and potentially delaying appropriate treatment, which may have severe consequences. Understanding factors associated with antimalarial treatment of malaria-negative patients is crucial to addressing this problem, and will be of even greater importance if malaria transmission decreases and the fraction of fevers attributable to malaria is reduced further. To understand current treatment practices and identify factors associated with antimalarial treatment of parasite-negative patients, we conducted surveys at 320 health facilities in three regions in Tanzania with varying malaria epidemiology (Mwanza, Mbeya, and Mtwara). Surveys were undertaken in 2010 and 2012 before and after nationwide roll-out of rapid diagnostic tests for malaria (mRDTs). Patients with fever in the previous 48 hours were interviewed following their consultation at the facility. Finger prick blood samples were taken by the study team to test for malaria parasitemia, allowing cross-referencing with any diagnostic test used by facility staff. Data were collected on patient characteristics, previous treatment for fever, and care received at the facility. Health workers were interviewed about their qualifications, training and supervision, knowledge, and facility stocks of antimalarials and mRDTs. At baseline, data was collected on 1739 patients (follow-up data collection ongoing). By study blood slides, 93% tested negative for malaria in Mwanza, 98% in Mbeya, and 79% in Mtwara. Overall, 42% of malaria-negative patients were treated with antimalarials by health facility workers. We will report the results of multivariate regression analyses accounting for the complex sample design to identify patient, health worker and facility-level factors associated with correct management of malaria-negative patients before and after widespread availability of mRDTs to support health worker decision-making. These results will be relevant to the success of mRDT roll-out and the design of interventions to reduce over-treatment of malaria.

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COMMUNITY LEVEL MANAGEMENT OF FEVER IN AFGHANISTAN - THE ROLE OF MALARIA RAPID DIAGNOSTIC TESTS

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In areas of low and seasonal malaria transmission, differential diagnosis of non-specific fever is important for patient care, control of malaria and in treatment and control of non-malarial causes of fever. Afghanistan is endemic for both *vivax* and *falciparum* malaria but with a low transmission intensity and dominated by *vivax* which accounts for 80-90% of cases. Our previous research has shown that malaria is consistently over-diagnosed and treated at the clinic level, but little is known about how community health workers (CHW) treat patients in the community. A cluster randomised trial of malaria rapid diagnostic tests (RDT) was undertaken using 400 CHWs to recruit 2600 patients in two transmission areas of Afghanistan. All CHWs administratively attached to 22 clinics (clusters) received training on management of malaria according to Government and WHO guidelines. Half of the clinics were randomly assigned to the intervention (RDTs), while half used clinical signs and symptoms for diagnosis and treatment. The primary outcome was the proportion of patients appropriately treated and aimed to evaluate whether the intervention resulted in improved targeting of treatment for patients with and without malaria. This included the use of artemisinin combination therapy for the rarely encountered cases of *falciparum* malaria. The outcome was measured against PCR based diagnosis of malaria to give a gold-standard diagnosis. The accuracy of the RDT and the prescribers' response to the results was assessed. This presentation will outline the results of the study and discuss implications for policy and practice of fever treatment at community and clinic level in malaria endemic areas outside Africa.

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ASSOCIATIONS BETWEEN FIVE DIAGNOSTIC METHODS OF PLACENTAL MALARIA AND LOW BIRTH WEIGHT IN AN AREA OF HIGH MALARIA TRANSMISSION IN TORORO, UGANDA

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The adverse consequences of placental malaria (PM) have been well established. Most studies rely on measures of PM as a surrogate marker of adverse birth outcomes such as low birth weight (LBW). However, there are no consistent standards for defining PM. Determination of a diagnostic standard is essential in order to compare studies using PM as the outcome of interest. This study compared the associations between 5 different definitions of PM and LBW. A total of 565 HIV-uninfected pregnant women were enrolled at delivery and infants were weighed at birth. LBW was defined as less than 2500 grams. Specimens collected included placental blood and tissue for histopathology. Placental blood was used for 3 definitions of PM: 1) positive blood smear (BS) for asexual parasites, 2) positive HRP2-based rapid diagnostic test (RDT), and 3) positive PCR. Placental histopathology was used for 2 definitions of PM: 1) any evidence of asexual parasites or hemozoin pigment, and 2) a quantitative assessment of hemozoin pigment (present in > 50% of 50 high powered fields examined.) The overall prevalence of PM defined by placental BS, placental RDT, placental blood PCR, conventional histopathology, and quantitative assessment of hemozoin pigment was 17.5%, 23.4%, 28.1%, 65.8% and 16.9%, respectively. Placental BS and RDT were significantly associated with LBW (RR=1.72, CI=0.99-2.99 and RR=1.97, CI=1.19-3.26, respectively) whereas placental PCR was not (RR=1.42, CI=0.72-4.18). Any evidence of PM by histopathology was not significantly associated with LBW (RR=1.73, CI=0.72-4.18); however, the quantitative assessment of hemozoin pigment was strongly associated with LBW (RR=3.60, CI=1.75-7.39). Placental histopathology was the most sensitive test for evidence of active or past placental malaria. Placental BS and RDT were also associated with LBW, while placental PCR was not. A definition of PM based on quantitative measure of hemozoin pigment from histopathology specimens may provide the best surrogate measure of adverse birth outcomes.

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DETECTION OF PLACENTAL MALARIA AND IMPACT OF RDT SCREENING AND TREATMENT ON PREGNANCY OUTCOMES IN AREAS OF VARIED TRANSMISSION

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The negative effects of malaria infection in pregnancy have long been recognized. Intermittent preventive therapy (IPTp) with sulfadoxine-pyrimethamine (SP) is becoming less effective as parasite resistance increases. New antimalarial medicines for IPTp are being considered, but have disadvantages. Therefore, screening with malaria rapid diagnostic tests (RDTs) may offer an accurate and practical way to identify pregnant women who will benefit from targeted antimalarial therapy. We assessed the association between antenatal (ANC) and intrapartum RDT results and pregnancy outcome in two African clinical settings (Uganda, hyperendemic, and Burkina Faso, seasonal transmission). We enrolled 995 (345 Uganda, 650 Burkina Faso) HIV-negative women in the second or third trimester of pregnancy and followed them to delivery. On the standard IPTp schedule and at the time of delivery, participants' blood was collected for RDTs, microscopy, PCR and hemoglobin measurement; placental tissue for histology was obtained at delivery. Participants with negative RDT results received SP; those with a positive RDT received artemether-lumefantrine or quinine, and SP. Preliminary data from Uganda show that 123 (45%) participants had positive RDT results at routine ANC visits. There was no significant difference in mean birth weight for mothers who had a positive RDT at time of usual IPTp dose and those who had all negative RDT results (3.10 kg versus 3.13 kg, $t=0.93$, $p=0.35$). There were 8 adverse pregnancy outcomes, 7 of which were among women whose RDT results were all negative and who received SP only. At time of delivery there was no difference in maternal hemoglobin (12.12 g/dL in those with RDT positive results and 12.35 g/dL in RDT negatives, $t=1.03$, $p=0.31$). Additional data will be presented from both sites on the accuracy of diagnostic testing for malaria during pregnancy and on the potential for malaria RDT screening and treatment of asymptomatic pregnant women during antenatal visits to impact pregnancy outcomes.

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TO ASSESS WHETHER INDOOR RESIDUAL SPRAYING CAN PROVIDE ADDITIONAL PROTECTION AGAINST CLINICAL MALARIA OVER CURRENT BEST PRACTICE OF LONG-LASTING INSECTICIDAL MOSQUITO NETS IN THE GAMBIA: A TWO-ARMED CLUSTER-RANDOMIZED STUDY

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Recently, there has been mounting interest in scaling-up vector control against malaria in Africa. It needs to be determined if indoor residual spraying (IRS with DDT) will provide significant marginal protection against malaria over current best practice of long-lasting insecticidal nets (LLINs) and prompt treatment in a controlled study, given that DDT is currently the most persistent insecticide for IRS. A two armed cluster-randomised controlled study was conducted to assess whether DDT IRS and LLINs combined provided better protection against clinical malaria in children than LLINs alone in rural Gambia. Each cluster was a village, or group of small adjacent villages. All clusters received LLINs and half received IRS in addition. 7,800 children, aged 6 months to 14 years, were enrolled and followed for clinical malaria using passive case detection to estimate malaria incidence, the primary endpoint, for two malaria transmission seasons in 2010 and 2011. Exposure to malaria parasites was assessed using light and exit traps followed by detection of *Anopheles gambiae* species and sporozoite infection rates. Children were surveyed at the start of the study and the end of each transmission season to determine *Plasmodium falciparum* parasite rates and prevalence of anaemia. Study findings will be discussed in relation to effective malaria control in the Sahel.

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UNDERSTANDING THE LOCAL POPULATION STRUCTURE OF *PLASMODIUM* IN THE CONTEXT OF MALARIA CONTROL AND ELIMINATION

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There is always population structure and malarial parasites are not an exception. The meaning of such structures, however, would depend on the scale and focus of the research. In the context of malaria control, the evaluation of treatment efficacy requires genotyping parasites at the local or regional level to distinguish homologous from heterologous parasites in recurrent infections as well as to get a good knowledge of the haplotypes circulating in the area. Unveiling such dynamics requires a good understanding of the temporal population structure and the accuracy of the information will depend on the adequacy of the markers used. In this study, we determined the minimum number of microsatellites needed to differentiate *Plasmodium* population clusters and haplotypes pertaining to each cluster in sympatry by using 215 blood samples (107 infected with *Plasmodium vivax* and 108 infected with *P. falciparum*) from a population in Tumeremo (Bolivar State) in Venezuela collected between March 2003 and November 2004. We found that malarial parasites undergo clonal expansions and that such dynamics needs to be taken into account during the onset of drug resistance at a local level. The use of this design could be easily applied in epidemiological studies to differentiate reinfection from recrudescence cases, describe gene flow and identify lineages that area stable in time. This information would be useful in determining specific geographic units of malaria treatment and control.

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EVALUATION OF THE EFFICACY AND SAFETY OF REDUCING DOSES OF PRIMAQUINE FOR CLEARANCE OF GAMETOCYTES IN UNCOMPLICATED *FALCIPARUM* MALARIA IN CHILDREN IN UGANDA

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Administration of the gametocytocidal drug primaquine (PQ) is a well-recognized tool to block transmission of malaria from humans to mosquitoes. The World Health Organization (WHO) has recommended adding a single dose of PQ to artemisinin-based combination treatment for *falciparum* malaria, particularly as a component of an elimination program. However, in individuals with glucose-6-phosphate dehydrogenase deficiency (G6PDd), PQ can cause life-threatening hemolysis, which has restricted its widespread use in regions where G6PDd is prevalent. This adverse effect is dose-dependent. We hypothesize that administration of PQ at a dose lower than that recommended by the WHO (0.75 mg/kg) will be safer than, yet as efficacious as, the WHO dose. We are currently conducting a randomized, double-blinded, placebo-controlled clinical trial to compare the efficacy and safety of three doses of PQ in Uganda. Children aged 1-10 years with uncomplicated *falciparum* malaria and normal G6PD status are recruited and treated with artemether-lumefantrine. On the third day of treatment, participants are randomized to receive 0.1mg/kg, 0.4mg/kg or 0.75mg/kg of PQ, or placebo. Participants are followed up for 28 days with repeated blood sampling. Efficacy outcomes include the number of days to gametocyte clearance (measured by quantitative real-time nucleic acid sequence-based amplification [QT-NASBA] on days 0-14, and the area under the curve of QT-NASBA-measured gametocyte density over time. Safety outcomes are the mean maximal change in hemoglobin on days 0-28, requirement for blood transfusion, evidence of hemolysis and incidence of adverse events. Efficacy analysis will be conducted for non-inferiority of each reduced dose of PQ treatment compared to the WHO-recommended dose. For safety, the superiority of test doses to standard dose will be assessed. Recruitment started end-December 2011 and 200 (42%) of the target sample size of 480 participants have been recruited to date. Complete, un-blinded results and full results of safety and tolerability will be presented.

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CLUSTER-RANDOMIZED 12-MONTH STUDY INVESTIGATING THE EFFECT OF COMMUNITY SCREENING AND TREATMENT OF ASYMPTOMATIC CARRIERS OF *PLASMODIUM FALCIPARUM* MALARIA WITH ARTEMETHER-LUMEFANTRINE ON SYMPTOMATIC MALARIA EPISODES IN CHILDREN AGED <5 YEARS AND HEMOGLOBIN LEVELS IN SUB-SAHARAN AFRICA

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The systematic detection of asymptomatic carriers (ACs) of *Plasmodium falciparum* by rapid diagnostic test (RDT) and subsequent treatment with artemisinin-based combination therapy has the potential to impact disease transmission. A single-center, controlled, parallel, cluster-randomized study was conducted in Burkina Faso to evaluate the impact at the community level of detecting and treating ACs during 3 community screening campaigns (CSCs) conducted before the rainy season. The two primary endpoints were: the number of symptomatic malaria episodes with a parasite density >5000/µL ($SMRC_{5000}$) per person-year in infants and children aged <5 years during the follow-up period, and hemoglobin (Hb) level change from day 1 to day 28 of CSC1 in treated vs. untreated ACs >6 months of age. 18 villages were randomized in a 1:1 ratio. In the intervention arm, ACs were identified by RDT and treated with artemether-lumefantrine (AL) or an alternative (if AL was contraindicated). Blood was collected in the intervention arm from all subjects and in the control arm from a randomly selected subset, at CSC1 for Hb measurement and at each CSC for delayed microscopy reading so that subjects (and study personnel) remained unaware of their AC status. Symptomatic malaria episodes were treated with AL or alternative in both arms for the duration of the study. In total, 14,075 subjects entered the study. At study end, the cluster-level mean number of $SMRC_{5000}$ per person-year in infants and children aged <5 years was similar between the intervention and the control arms (1.69 vs. 1.60; *P=0.3482*). The mean change in Hb levels from day 1 to day 28 of CSC1 in the intervention arm was 0.53 g/dL vs. -0.21 g/dL in the control arm (*P*<0.0001). These results show that the systematic detection by RDT and treatment of ACs at the community level did not have a significant impact on disease transmission in the population. Although the change in Hb level between the two arms was statistically significant, it was not deemed to be clinically meaningful.

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THE EFFECTIVENESS OF A SINGLE ROUND OF MASS MALARIA SCREENING AND TREATMENT IN SOUTHERN ZAMBIA

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In Zambia the current interventions of insecticide-treated mosquito nets, indoor residual spraying and case management with artemisinin combination therapy are not likely to result in malaria elimination alone. As part of a pilot mass malaria screening and treatment intervention, 10 health facilities in Gwembe and Sinazongwe districts, Southern Province, Zambia were randomly selected to receive a single round of mass malaria screening and treatment preceding the 2012 high malaria transmission season. In December 2011 and January 2012 approximately 50,000 individuals, regardless of symptoms, were tested for malaria parasites by community health workers using ICT Mal Pf rapid diagnostic tests. Individuals testing positive were treated with artemether-lumefantrine, the national first line malaria treatment. The single round of mass malaria screening and treatment will be evaluated using a combination of study designs and analyses: 1) a randomized post-only comparison between intervention and control areas of parasite prevalence in children < 6 years of age measured through an oversampled malaria indicator survey in April 2012; 2) a randomized post-only comparison between intervention and control areas of parasite prevalence in all individuals measured through the first round of the intervention in June 2012; 3) a pre-post comparison of parasite prevalence within intervention areas (follow-up June 2012); and 4) a randomized longitudinal comparison of monthly outpatient laboratory-confirmed malaria cases recorded from health facilities within the 2 districts. Each method of evaluation has limitations including the lack of a baseline in the randomized post-only comparisons, the lack of a counterfactual in the pre-post comparison, and known biases in health facility routine data. Preliminary results will be available in September 2012.

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REACTIVE CASE DETECTION FOR MALARIA ELIMINATION: REAL-LIFE EXPERIENCE FROM AN ONGOING PROGRAM IN SWAZILAND

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Reactive case detection, whereby households and neighbors of passively detected cases are screened and treated, is being implemented in a number of *Plasmodium falciparum* malaria eliminating countries. This type of surveillance is designed to take advantage of the spatial clustering of infection and is primarily used to target the asymptomatic infectious pool. A crucial, yet not well understood, aspect of reactive case detection is the size of the screening radius employed around each index case. Using nationwide reactive case detection data from Swaziland, collected between December 2010 - March 2012, analyses were conducted to explore the relationship between the probability of detecting a case and distance to the passively detected index case. Results show that infection is highly clustered at the household level, with the odds of detecting a secondary case being six to seven times higher within the index household than in households located either up to 100m or over 100m away. The probability of detecting a case outside the index household did not appear to be associated with whether another case was detected inside the index household. From an operational perspective, the data suggest that using a screening radius of 1km is extremely challenging with high coverage unlikely to be achievable in resource constrained settings. Together, these results suggest that future reactive case detection in Swaziland could be made more efficient by focusing screening on a smaller radius or on the index household itself.

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ALLOCATIVE EFFICIENCY MODELING OF MALARIA TREATMENT AND PREVENTION: ANALYSIS OF INTERACTIONS TO IMPROVE INTERVENTIONS

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The recent expansion of anti-malaria efforts and increased focus on local elimination have resulted in greater funding and enhanced distribution aimed at reducing disease burden. Both donors and public health agencies have become interested in how to maximize impacts of various kinds of interventions. Not surprisingly, allocation of resources toward different types of treatment and prevention may have complex effects on the dynamics of transmission and effectiveness of disease suppression. Despite the absence of a singular model for successful control, there is a need to understand how introduction of interventions that bundle into a given system results in observed outputs and outcomes to predict the optimal deployment of resources. We developed a system dynamics, compartmental, simulation model of population transition among states of parasitemic and/or symptomatic as well as susceptible and/or infectious. Age-specific survival and entomological inoculation rates were drawn from the literature. Different allocations of four interventions (prevention with ITNs, IRS and IPT, and treatment with ACT) produced very different impacts on population patterns of transmission, infection and disease across different transmission contexts. Exemplary results are presented in the context of algorithms that could be aimed at improving effectiveness of such large-scale intervention. The ultimate goal of our efforts is to produce a user-friendly program that will allow countries to better understand the complex interactions that malaria reduction efforts produce, and in so doing provide policy makers with the tools to improve resource allocation.

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THE ROLE OF *LUTZOMYIA INTERMEDIA* SANDFLY SALIVA ON THE EARLY EVENTS OF *LEISHMANIA BRAZILIENSIS* INFECTION

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Leishmania parasites are transmitted to the mammalian hosts by the bite of phlebotomine sandflies. During this process, not only parasites but also sandfly salivary products are delivered to the host. *Leishmania braziliensis* is the etiological agent responsible for cutaneous and mucocutaneous leishmaniasis throughout Brazil and the parasite is transmitted by *Lutzomyia* genus of sandflies. The objective of this study was to investigate the influence of sandfly saliva on the development of the immune response to *L. braziliensis* infection. Previously, immunization with sandfly salivary gland extract (SGS) from *L. longipalpis* was shown to protect against infection while the opposite effect was observed following *L. intermedia* preimmunization. To understand the mechanisms involved in these differences, we analyzed the impact of *L. intermedia* preimmunization on the innate immune response. First, we characterized the cellular infiltrate in response to SGS inoculation in the presence or absence of parasites. Next, we examined the cellular recruitment and gene expression profiles in mice preimmunized with *L. intermedia* compared to mice given PBS as a control. The global effect of preimmunization with *L. intermedia* SGS on gene expression was subjected to microarray analysis, revealing a distinct set of IFN-inducible genes that were upregulated in response to immunization with SGS; however, these genes were silenced at the time analyzed in mice given *L. braziliensis* suggesting the parasite is modulating the dermal microenvironment creating a niche for parasite persistence.

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DEMONSTRATION OF REPRODUCIBLE VISCERALIZATION OF *LEISHMANIA DONOVANI* FOLLOWING TRANSMISSION BY SAND FLY BITES IN BALB/C MICE AND HAMSTERS

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Visceral leishmaniasis (VL) caused by *Leishmania donovani* is a vector-borne anthroponotic disease transmitted by sand fly bite with no available human vaccines. Following vaccination, animals protected against cutaneous leishmaniasis upon needle challenge failed against the virulence of a sand fly-initiated infection. This highlights the significance of developing models of vector-transmission for VL, particularly in vaccine evaluation. Here, we present models of visceral leishmaniasis in BALB/c mice and Golden Syrian hamsters using *Lutzomyia longipalpis* sand flies infected with *L. donovani*. Sand flies with transmissible infections were allowed to feed on animal ears for 2 hours. In BALB/c mice, most animals developed *Leishmania*-specific IgG antibodies around 5 weeks post-infection. Ten weeks post-infection, the parasites had disseminated into the spleen and liver reaching a maximum burden of $1x10^6$ and $2x10^3$ in the spleen and liver, respectively, at 20-25 weeks. Thirty weeks post-infection, the mice had not cleared the infection displaying a significant number of parasites ($6.5x10^4$) in the spleen although none were detectable in the liver. The progressive growth of parasites in the spleen and liver of infected mice following vector-initiated infection demonstrates the utility of this model to study VL. In hamsters, the animals succumbed to disease within 3-9 months post-sand fly transmission showing parasite visceralization accompanied by clinical manifestations of VL including enlarged spleens and livers whose mean weight was 5.8- and 1.3-fold higher, respectively, than those of naïve hamsters. Currently, studies in BALB/c mice and hamsters are focusing on the comparative evaluation of the immune response following infection with either sand fly bite or needle injection and also are oriented towards testing promising vaccines that protected against needle-challenge. Overall, these models facilitate our understanding of the host immune response to vector-initiated VL and represent an improved tool for the assessment of potential drugs and vaccine candidates.

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HUMAN AFRICAN TRYPANOSOMIASIS RESERVOIR STATUS IN TANZANIA

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Human African Trypanosomiasis (HAT) is transmitted by *Glossina* species. Livestock and wildlife play an important role in maintaining the disease as reservoirs. A study was conducted to assess the reservoir status of livestock from three sites in Tanzania namely north western near the Serengeti ecosystem, western zone (Ugalla) and Rufiji in the south. The study was conducted during the onset of dry season of 2010 and 2011. From Serengeti ecosystem, 150 cattle were screened and only one cattle was parasitological positive, and by PCR, the infection rate by the *Trypanosoma brucei* types were 5/150 (3.3%) and all were SRA LAMP positive. From western zone screening involved 574 cattle, 108 Goats and 21 Dogs.

Parasitological positive were 34/574 cattle, 2/108 goats and 1/21 dogs. PCR analysis recorded an infection rate of 66/300 (22%) in cattle, for *T brucei* and 30 (45.4%) of 66 were positive by SRA LAMP. PCR analysis recorded 16/108 (14.8%) infection for *T. brucei* but negative by SRA LAMP in Goats. PCR recorded 4/11 *T. brucei* infections in dogs which were all negative by SRA LAMP. From Southern Tanzania, a total of 404 animals were screened which included 202 cattle, 85 sheep, 10 dogs, 5 donkeys and 102 goats. Microscopic analysis recorded 4 animals infected by *T. congolense* types of trypanosomes. PCR analysis of 69 animal blood out of 404, recorded 38/69 (55%) positive for *T. brucei* types. All animal species were equally affected by the *T. brucei* types as infection in cattle was 33/48 (68.7%); 2/6 for sheep and one each (1/5) for donkeys, goats and dogs. All *T. brucei* positive samples from southern Tanzania were negative by SRA LAMP. Results from this study indicate that livestock especially cattle may play an important role in the epidemiology of HAT and control of the vector, *Glossina,* supplemented with treatment of animals is an important measure for control of human infective trypanosomes, and HAT epidemiology. Of importance is the finding that many animals were positive for *T. brucei*, which in some cases like northern and western sites could be of zoonotic importance as confirmed by SRA LAMP. Presence of human serum resistance associated (SRA) gene confirms the presence of human infective trypanosomes that cause the *T. brucei rhodesiense* form of HAT.

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TOWARDS *TRYPANOSOMA CRUZI* LINEAGE-SPECIFIC SEROLOGY FOR CHAGAS DISEASE

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Chagas disease, caused by the protozoan *Trypanosoma cruzi*, remains an important parasitic disease in the Americas. It can be fatal in the acute phase, but life-long chronic infection may be asymptomatic, or lead to debilitation and death by cardiac and/or intestinal complications. Genetically diverse, *T. cruzi* is classified into the intra-species lineages TcI-TcVI, displaying disparate geographical distributions and ecologies. The varying disease outcomes may be linked to parasite lineage, and complicated by mixed infections. The work presented here addresses the development of lineage-specific serology to identify an individual's history of exposure to *T. cruzi* lineages. The molecular diversity of the parasite surface antigen TSSA was analysed across a panel of reference biological clones encompassing *T. cruzi* genetic and ecological diversity, revealing lineage-specific B-cell epitopes. We demonstrate here the capacity of synthetic peptides based on the TcII/V/VI common epitope to be recognised by antibodies in human sera from Brazil, Chile, and reported for the first time, Ecuador. Further, we report the first TcIII- and TcIV-specific serology, from experimental murine models. A genomic approach to identify *T. cruzi* lineage-specific epitopes can be used successfully in developing a differential serology to investigate an individual's history of *T. cruzi* lineage exposure, and lead to a greater insight into the link with Chagas disease outcome. Overall, this approach represents a potential new tool in Chagas disease epidemiology.

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COMPARATIVE GENOMICS AND PHYLOGENOMICS OF THE PROTOZOAN PATHOGEN, *TRYPANOSOMA BRUCEI*

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The protozoan pathogen, *Trypanosoma brucei* is the causative agent of Human African Trypanosomiasis (HAT) which affects mainly poor rural populations across sub-Saharan Africa. *T. brucei* is separated into three subspecies based on the disease forms they cause: *T. b gambiense* - which causes a chronic form of HAT, *T. b. rhodesiense* - which causes an acute for of HAT and *T. b. brucei* - which causes the livestock wasting disease Nagana. We conducted whole genome sequencing of 16 isolates from across the distribution of *T. brucei*, followed by a referenced alignment to the annotated TREU927 T. brucei genome and identified 352,505 single nucleotide polymorphisms (SNPs) across the genome. Selection and repetition were estimated to provide a comparative genomic framework across the *T. brucei* genome. In addition, to test the validity of subspecies designations and competing evolutionary hypotheses in *T. brucei*, we developed a phylogenomic framework of 9,500 neutrally evolving, independent and unique sequence loci from 500 - 5,500 base pairs in length. Using species tree methods, we estimated the phylogeny of the *T. brucei* complex to determine relationships between *T. brucei* subspecies and identify the ancestral lineage within the species complex.

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ROLE OF THE CHROMATIN REMODELING ENZYME HDAC1 IN *LEISHMANIA AMAZONENSIS* INFECTION: IMPLICATIONS FOR HOST TRANSCRIPTION REPRESSION

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Leishmania parasites subvert important host cell signaling pathways involved in the control of the infection. NF-κB is an important transcriptional factor which modulates the expression of genes involved in the immune response. Recent results from our group demonstrated the activation of NF-κB transcriptional repressor homodimer (p50/p50) in *L. amazonensis*- infected macrophages, treated or not with LPS. As a result of this homodimer complex activation, we observed the down-regulation of the expression of nitric oxide synthase (iNOS) in infected macrophages treated with gamma Interferon. Besides the activation of transcriptional factors, chromatin epigenetic modifications are pivotal regulators of gene transcription. Chromatin remodeling proteins such as deacetylase histones (HDAC) are involved with transcriptional repression and may be associated with transcriptional factors, forming large repressor complexes. In this work, we have studied in details the iNOS transcriptional repression during *L. amazonensis* infection through the analysis of iNOS promoter occupancy by p50/p50 NF-κB complex and the participation of HDAC 1 in these events. We have found that the increased occupancy of p50/p50 iNOS promoter depends on PI3K/Akt pathway in *L. amazonensis* infected cells. Consistent with transcription repression, we have detected an increase in HDAC1 mRNA and protein levels, as well as an increased activity of total histone deacetylase in infected macrophages. We have verified a relevant reduction of *L. amazonensis* amastigote growth in macrophages silenced for HDAC1 expression. We also verified the mRNA iNOS increased levels in infected macropages during HDAC1 silencing, showing the participation of this deacetylase in iNOS promotor regulation. In fact, we have observed an increased occupancy of HDAC1 in NF-κB promoter-binding site and a decreased occupancy of acetylated histone 3 (Lys 9). These results indicate that important epigenetic modifications associated with p50/p50 NF-κB homodimer are taking place in infected macrophages.

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MONOCYTE-DERIVED TNF-A AND METALLOPROTEINASE 9 IN PATIENTS WITH CUTANEOUS LEISHMANIASIS

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Cutaneous leishmaniasis (CL) caused by *Leishmania braziliensis* is characterized by the presence of one or more ulcerated lesions with elevated borders. High levels of IFN-γ and TNF-α are detected in these patients and these proinflammatory cytokines are known to play a role in the pathogenesis of CL, by inducing tissue damage. Upon infection with *Leishmania* or in presence of SLA, monocytes from CL individuals produce high levels of TNF-alpha involved in recruitment of monocytes. Recent studies have shown that circulating monocytes constitute a heterogeneous population based on expression of CD14 and CD16, these cells can be divided in classical (CD14+CD16-), intermediate (CD14+CD16+) and non-classical (CD14-CD16+) monocytes. Intermediate and non-classical monocytes are known to migrate to inflamed sites and secrete inflammatory mediators, and high frequency of these cells has been associated with pathogenesis of many inflammatory diseases. TNF-α can mediate the pathology of the disease through various mechanisms including induction of nitric oxide, expression of metalloproteinases (MMPs) and increased cytotoxicity. MMP-9 is a zinc-dependent enzyme that degrades collagen and has been associated with skin inflammatory diseases. Although the mechanism underlying ulcer development in CL is not known, it´s likely that MMP-9 contribute to tissue damage. Thus, our goal was to investigate the contribution of sub-populations of monocytes to TNF-α and MMP-9 secretion in CL patients. We found that early after infection (pre-ulcerative phase) the frequency of intermediate and non-classical monocytes is elevated in blood of CL patients. Also, while intermediate monocytes produced more TNF- α in response to *Leishmania*, non-classical ones were the main source of MMP-9 in most CL patients. Similarly, the biopsies study reveled that non-classical monocytes were the main MMP-9 producing cells. These results show that monocytes subpopulations contribute differently to the immunopathology observed in CL patients.

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UTILITY OF A *WUCHERERIA BANCROFTI* SPECIFIC WB123-BASED IMMUNOASSAY FOR USE AS A SURVEILLANCE TOOL FOLLOWING CESSATION OF MASS DRUG ADMINISTRATION IN A *W. BANCROFTI* -ENDEMIC AREA OF MALI

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Significant progress has been made toward the global goal to eliminate lymphatic filariasis (LF) by 2020 though the tools for monitoring control success and certification of transmission interruption need to be refined. Recently modified WHO guidelines for transmission assessment surveys (TAS) have recently been proposed to guide decisions about stopping mass drug administration (MDA), but the tools for post MDA surveillance are likely to involve antibody testing. To assess the utility of antibody testing in a target (6-7 year olds) population, we assessed antibody reactivity to Wb123, a *Wuchereria bancrofti* (Wb)-specific antigen that is expressed early in parasite development and has been shown to be a sensitive and specific marker of exposure to Wb infective stage larvae (L3). Wb123 antibody was compared to calibrated thick smear of midnight blood. 298 children 6-7 years old from two villages in Mali one year following the cessation of 5 rounds of MDA were assessed. Using bloodspots for Wb123 antibody levels and a Wb123 luciferase immunoprecipitation assay systems (LIPS), only 1 of the 298 (0.3%) children tested were positive for anti-Wb123 antibody; a similar prevalence was seen on night blood smears (Wb microfilaria prevalence 0.3%). These data suggest that Wb123-specific antibody testing in children can be a sensitive and specific tool for monitoring transmission following MDA cessation. Given the prevalence of 0.3% (well less than 1%) continued yearly follow-up of prevalence in 6-7 year olds will provide insight into the continued utility of Wb123 immunoassays for Wb transmission assessment following MDA not only in this area of Mali, but throughout Africa where co-incident filarial infections have limited the use of other recombinant antigen-based immunoassays.

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HOST CHOICE BY ONCHOCERCIASIS VECTORS AND ONGOING TRANSMISSION IN AREAS UNDER IVERMECTIN CONTROL

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The ability of mathematical models to predict intervention impact on vector-borne diseases will be affected by whether the proportion of bloodmeals taken on humans depends on vector and/or host density. Empirical data on onchocerciasis transmission and vector host choice in areas which have received prolonged vector control and mass annual ivermectin treatments will enable locality- and vector-specific prediction of *Onchocerca* transmission. Seven study sites in four regions of Ghana were visited from 2009 to 2011 in both rainy and dry seasons, to study variation in blackfly and host densities, and host choice. Surveys of wild birds and mammals, households and domestic animals were conducted. Blackflies (15,466; 85% *Simulium damnosum* s.l.) were collected by host-dependent and host-independent methods, assessed for parity, and stored for molecular and morphological analysis for identification of fly and *Onchocerca* species, and bloodmeal origin. The size of human populations varied from 188 to 5,202; of domestic animals from 489 to 11,143; and the number of bird species from 31 to 61. Blackfly biting rates ranged from 0 to 298 bites/person/day, and parity rates from 18 to 27% (wet season) and from 30 to 46% (dry season). Three of the villages had levels of L3 larvae/1,000 parous flies above the WHO threshold for morbidity and transmission control (range 1.4 to 115.1 L3/1,000 parous flies) despite annual distributions of ivermectin for up to 23 years in one village. In these villages exposure to infective L3 larvae was between 0.04 and 3.66 L3/person/day. Flies had fed on a range of hosts, predominantly humans and pigs. *Onchocerca* spp. other than *O. volvulus* were recorded. Results indicate that blackflies have multiple blood hosts and that active transmission is still occurring despite annual or biannual ivermectin treatment. Such data will inform control programmes about the feasibility of, and the duration of ivermectin treatment required for, elimination of onchocerciasis.

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MOSQUITO-PARASITE INTERACTIONS AND IMPLICATIONS FOR FILARIASIS TRANSMISSION IN PAPUA NEW GUINEA

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In Papua New Guinea, filariasis is transmitted by members of the *Anopheles punctulatus* group of mosquitoes while culicines, major vectors in neighboring regions of the Pacific, are considered unimportant for transmission. In a diverse vector environment such as Papua New Guinea, it is likely that not all species contribute equally to transmission. Transmission potential can be influenced by vector competence to W. bancrofti as well as vector-host and vector-parasite interactions. To test this hypothesis, we exposed multiple vector species to microfilaremic blood of varying densities to measure time to development of infective-stage larvae (L3s) as well as prevalence and intensity of infection. At lower mf densities (30-60 mf/20ml) 30% of *An. farauti* s.s. harbored L3s (mean intensity 2.0) and 7% of *An. punctulatus* harbored L3s (mean intensity 1.0). At higher densities (130-160 mf/20 ml), 83% of *An. farauti* and 28% of An. punctulatus were permissive to the development of L3s with a mean intensity of 4.6 and 3.1 respectively. The extrinsic incubation period was equal in both species. In *Culex annulirostris*, no L3s were observed and development was halted at the first or second larval stage. To put this into the context of transmission in Papua New Guinea we also investigated mosquito parity rates, mosquito biting behavior and availability of microfilariae in peripheral blood. Both *An. farauti* s.s. and *An. punctulatus* had comparable age structures. Although An. farauti s.s. is a more competent vector, this species might have a lower capacity to transmit filariasis because of asynchrony between peak biting times and *W. bancrofti* periodicity. An. farauti s.s. has a peak biting time of 1900h, five hours earlier than the peak density of circulating microfilaria. As a result, An. farauti s.s. is exposed to approximately 33% of the mf that are available at the peak biting time for *An. punctulatus*. Filariasis elimination efforts can be greatly enhanced by the integration of vector control; however, a greater understanding of the influence of vector behaviors and vector-parasite dynamics on transmission is necessary to inform these strategies.

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THE COST-EFFECTIVENESS OF DOXYCYCLINE THERAPY FOR THE CONTROL OF HUMAN ONCHOCERCIASIS IN AREAS CO-ENDEMIC WITH LOIASIS

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The control of onchocerciasis in Africa is based on annual or biannual community-directed treatment with ivermectin (CDTI). However, CDTI is contraindicated in areas where loiasis is co-endemic, including large parts of central Africa, because of the risk of severe adverse effects (SAEs) associated with rapid microfilarial killing and blockage of brain vasculature leading to encephalopathy. An alternative strategy in these areas is to treat with doxycycline, which given daily for 4-6 weeks, is macrofilaricidal against *Onchocerca volvulus*, causing sustained reductions in adult worm and microfilarial loads. Crucially, *Wolbachia* is not present in *Loa loa,* which mitigates the risk of SAEs. Furthermore, the feasibility of achieving high levels of coverage and compliance with a six-week course of mass-distributed doxycycline and its long-term impact has been demonstrated in Cameroon. An onchocerciasis transmission model (EpiOncho) is used to show that community-directed treatment with doxycycline (CDTD) is approximately twice as effective in preventing cases of *O. volvulus* infection compared with CDTI and more than twice as effective in reducing levels of transmission. Moreover, CDTD is about as cost-effective as CDTI in loiasis co-endemic areas. This is partly because CDTD can be delivered less frequently than CDTI, and partly because of the high cost associated with implementing the additional monitoring and surveillance components of CDTI, which attempt to minimise the occurrence and impact of SAEs. We conclude that CDTD is a safe, viable and cost-effective alternative to CDTI for the control of onchocerciasis where loiasis is co-endemic.

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ARE FIVE ROUNDS OF ANNUAL MASS DRUG ADMINISTRATION NECESSARY FOR LYMPHATIC FILARIASIS (LF) TRANSMISSION INTERRUPTION? LA TORTUE, HAITI 2012

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The World Health Organization recommends five years of annual mass drug administration (MDA) in areas where Lymphatic Filariasis (LF) prevalence exceeds 1%, followed by a Transmission Assessment Survey (TAS) in 6–7 year olds to determine if transmission has been interrupted. It is not clear if 5 rounds of MDA are necessary where the initial antigen prevalence is >1%, but <10%. LF mapping performed in Haiti in 2001 showed that 73% of country's implementation units (IUs) fell within this prevalence range. For a country with limited resources, guidelines stating that <5 rounds of MDA are ample in low prevalence areas would allow for a re-evaluation of transmission within the vast majority of the country. This may result in stopping MDA, and refocusing efforts and resources on the remaining 27% of the IUs. In 2002 the prevalence of LF on La Tortue, Haiti was found to be 6%, and 2 rounds of MDA were performed, ending in 2005. A follow-up convenience sample of >1600 persons of all ages in 2006 found a prevalence of 0.6%. We performed a modified school-based TAS in 11–12 year old children on La Tortue to determine if transmission has been interrupted. Using Survey Sample Builder, we calculated a sample size of 909 children from 32 randomly selected schools with a critical cut-off of 11. After informed consent, we collected blood for ICT and filter paper testing, and GPS, demographic, migration, and risk factor data. We sampled 1082 children from 29 schools. A total of 7 children were positive for LF (prevalence 0.645%, 95% confidence interval: 0.389–0.901), below the critical threshold for transmission. Of the 7 positive children, only 1 child had migrated to the island. Data are being collected from the final three schools and filter papers are being run for antibody testing. Our results suggest that LF prevalence on La Tortue is below the accepted threshold for transmission interruption, and MDA does not need to be restarted. These findings suggest that 5 rounds of MDA may not be necessary in areas of Haiti where the initial prevalence was <10%.

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OPTIMISM FOR LYMPHATIC FILARIASIS ELIMINATION: A CASE STUDY OF TANDAHIMBA DISTRICT, SOUTHERN TANZANIA

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Lymphatic Filariasis is endemic in almost all districts in Tanzania. The National Lymphatic Filariasis Elimination Program strategy includes interruption of transmission via Mass Drug Administration (MDA) and morbidity control. Tandahimba District had five annual rounds of MDA with Mectizan and Albendazole, and the coverage was above 65% at each round. Baseline data were collected in 2002, and sentinel site data were collected after three and five rounds of MDA in 2006 and 2008, respectively. Results from four sentinel sites indicated that the microfilaria (mf) prevalence decreased from 6.8% before MDA to 0.4% after five rounds of MDA. As part of a multi-country survey, a first Transmission Assessment Survey (TAS) was conducted in 2009 by following the newly developed Global Guidelines for Monitoring and stopping MDAs.In 2011, two years after stopping MDA, a second TAS was conducted. Sampling for TAS was based on Enumeration Areas (hamlets), with a cluster-sample household survey of 6-7 year-old children. Circulating filarial antigens (CFA) were detected using Immunochromatographic Test cards (ICT).Each positive ICT case was traced and examined at night for Microfilaremia (mf). In 2009, a total of 1558 children from 69 hamlets were tested for CFA. Ten (0.64%) were positive and only one of these (10%) was mf positive. The follow-up TAS conducted in 2011 involved 1605 children and only 9 (0.56%) were ICT positive. None of these were night blood mf positive. The findings from the TAS surveys indicated that the ICT prevalence was well below the critical cut-off value of 2% for stopping MDA,as defined in the new WHO guidelines. On the basis of the significantly decreased LF transmission in Tandahimba District, it was decided to discontinue MDA and to intensify surveillance. This is the first district in Tanzania to have reached the critical cut off point for stopping MDA

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GOOD PROGRESS TOWARDS THE ELIMINATION OF LYMPHATIC FILARIASIS IN BANGLADESH

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Bangladesh has a long history of lymphatic filariasis (LF) caused by the parasite *Wuchereria bancrofti*, and is estimated to have 70 million people at risk of infection, with up to 10 million suffering from various forms of clinical deformity. The National LF Elimination Programme was one of the first to start the elimination process in 2001 with mass drug administration (MDA) using albendazole and diethylecarbamazine (DEC) in endemic areas. Of the 19 districts implementing MDA, five districts have received >5 rounds of MDA and sentinel sites have shown <1% microfilaria (Mf). Therefore, to determine if transmission has been interrupted, the new WHO Transmission Assessment Survey (TAS) was carried out in Meherpur, Barguna, Patuakhali, Rajshahi and Dinajpur districts. A school based survey was undertaken with 6 and 7 year old children as the target population. LF prevalence was measured using Immunochromatographic test (ICT), with sample sizes and critical cut off numbers calculated using the Sample Survey Builder. The TAS was carried out over a two month period, using trained field teams. The number of children sampled ranged from 1556 to 1692, with cut offs of 18 and 20 respectively. In total 9 children were found to be ICT positive. No positive cases were found in Meherpur and Patuakhali, however, seven positive cases were found in Dinajpur and one positive case in both Barguna and Rajshahi districts. These results indicate that LF transmission has been interrupted and MDA can stop in these districts. This success may be attributed to high MDA coverage facilitated by Government support, timely and coordinated efforts of the Programme and successful partnerships. This is promising for Bangladesh, however, it will be critical to develop and maintain a systematic post-MDA surveillance strategy to fully confirm the interruption of transmission and reach its goal of LF elimination by 2015.

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COMPARISON OF HEALTH FACILITY AND COMMUNITY-BASED ESTIMATES OF SOIL TRANSMITTED HELMINTH INFECTION IN NUEVA SANTA ROSA, GUATEMALA - 2010

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Soil transmitted helminth (STH) infections are associated with significant morbidity as well as decreases in cognitive function and growth retardation. In most countries with a significant STH burden, school-age children (SAC) are at greatest risk of infection, but preschool age children (PSAC) may also be infected. Data on STH prevalence by age is lacking for most of Guatemala and there is little information on PSAC. Data on community prevalence of STH is generally gathered through community-based surveys, but these are expensive and coverage is limited. We explored the use of STH prevalence data generated from stool testing for diarrhea surveillance in Guatemala as a proxy for community-based surveys. Sentinel surveillance for diarrhea ($\geq$ 3 loose stools in a 24 hour period) is conducted in selected peripheral health facilities in the county of Nueva Santa Rosa (NSR). For comparison, we estimated community prevalence between July and August 2010 by collecting stool samples from residents ≥ 1 year of age in randomly selected households in NSR, irrespective of their history of diarrhea. Stool samples were tested for *Ascaris sp., Trichuris sp.,* and hookworm using the Kato-Katz method. Individuals positive for any of the three parasites were considered infected. Facility-based surveillance data included 643 stool samples from 776 cases of diarrhea in 2010; 19 (3%) were positive for STH. Facility-level prevalence was highest among SAC (6%, 6/98), though 4% (10/267) of PSAC and 2% (3/135) of adults were also infected. The community survey included 324 residents, and 41 (13%) were infected with STH. Prevalence was highest among SAC (18%, 19/104), though 13% (7/54) of PSAC and 9% (15/166) of adults also tested positive. A larger proportion of the health facility STH cases were in PSAC compared to the community survey (53% vs. 17%) and the age distribution was significantly different (P=0.01). Our findings indicate that the use of stool samples from health facility patients presenting with diarrhea underestimates community-level STH burden and skews the age distribution of cases towards PSAC, possibly because more young children were brought to health facilities for diarrhea. However, both data sources indicate that both PSAC and SAC should be targeted for treatment to prevent negative outcomes associated with STH infection. Furthermore, consideration should be given to treating adults as possible reservoirs of household infections.

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SOIL-TRANSMITTED HELMINTHS IN URBAN SCHOOL- AND PRE-SCHOOL-AGED CHILDREN: PREVALENCE AND MORBIDITY IN KIBERA, NAIROBI

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Soil-transmitted helminth (STH) control programs face an increasing need to assess urban transmission. At the same time, while most prevalence studies and World Health Organization (WHO) deworming recommendations focus on school-aged children (SAC), STH burden and potential treatment benefit among pre-school-aged children (PSAC) are less known. We conducted a study of pediatric STH infection prevalence and morbidity in the Kibera informal settlement in Nairobi, Kenya. 899 SAC (5-14 years) and 293 PSAC (6-59 months) were randomly selected as index children from the enrollment registry of the community-based surveillance platform run by the CDC's International Emerging Infections Program in Kibera. Data from index children include a target of 3 stools tested by the Kato-Katz method for STH ova, anthropometry, hemoglobin, and family-reported febrile, diarrheal or respiratory illness. For SAC, sibling stools were tested for STH. Results from subjects with at least 1 stool (n=493 PSAC, 1225 SAC) were analyzed for STH prevalence and differences between age groups. In index children (n= 212 PSAC, 509 SAC), any STH infection was tested for correlation with anemia (based on age and altitude hemoglobin cutoffs as per WHO) and moderate or severe stunting, wasting or underweight as per WHO. All statistical testing was by chi-square. Prevalences were: any STH 42.3% (PSAC 39.0%, SAC 43.6%, p=.08); *Ascaris* 25.3% (PSAC 25.8%, SAC 25.1%, p=.76); *Trichuris* 27.8% (PSAC 21.9%, SAC 30.1%, p<.01); hookworm <0.1%; any co-infection 10.9% (PSAC 8.7%, SAC 11.8%, p=.06). Prevalence of anemia was 20.9% and of any malnutrition, 21.7%; no correlations with STH infection were found. STH infection is common in this population. PSAC and SAC have similar STH infection prevalences and both should be considered in control plans. Assessment of sibling STH infections as risk factors for index child infection and correlation of STH infection with micronutrient deficiencies and reported child illnesses will be discussed.

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SCREENING FOR *STRONGYLOIDES* INFECTION IN AN IMMIGRANT POPULATION IN BRONX, NEW YORK

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Chronic infection with Strongyloidiasis may transform into a fatal illness as a result of immunosuppression or HTLV-1 co-infection. The aim of this study was to define whether a routine screening program using serology for *Strongyloides* in immigrants from endemic regions is beneficial. Screening was conducted from 2004 to 2012, in inpatient and outpatient settings, at Jacobi Medical Center, Bronx, New York. *Strongyloides* serology was performed by serum ELISA. Blood cell counts performed in all patients. If a positive serology was detected, IgE level, HTLV-1 serology and stool ova/parasite were performed when feasible. A total of 631 individuals (317 male [50.2%]) were screened, mean age of 56±17 years. No differences related to age/sex were found between sero-positive and -negative patients The majority of patients were from Puerto Rico (21.9%), Jamaica (15.4%), Dominican Republic (6.5%), Mexico (5.4%), Guyana (4.4%), Bangladesh (3.8%), Ecuador (3.6%). Eighty-nine percent of patients were screened during inpatient admissions. IgG antibodies were detected in 86 (14%) patients. Mean time from immigration was 25±18 years, with no difference between the 2 groups. There was no difference regarding history of walking barefoot in home country, asthma, steroid use, complaints of abdominal pain or skin rash. Ser-positive patients were more likely to have eosinophilia (absolute count >500 cells/ml) compared to ser-negative patients (p<.001). Elevated IgE level (mean 522±634 UI/ml) was observed in 32 (67%) patients who screened positive; 16/26 (62%) sero-positive patients with a normal eosinophil count had elevated IgE levels. Stool exams were performed in 51/86 positive patients and 6 had larvae. Co-infection with HTLV-1 was found in 4/57 (7%) sero-positive patients. Those with positive serology were more likely to have eosinophilia and 62% of those without eosinophilia had elevated IgE. Immigrants with either eosinophilia or elevated IgE are candidates for routine screening, despite long-term residency in the USA.

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A NOVEL MULTI-PARALLEL REAL-TIME PCR APPROACH FOR EIGHT GASTROINTESTINAL PARASITES PROVIDES IMPROVED DIAGNOSTIC CAPABILITIES TO RESOURCE-LIMITED "AT RISK" POPULATIONS

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Diagnosis of gastrointestinal parasites has traditionally relied on stool microscopy that has low diagnostic sensitivity and specificity, is time consuming and labor intensive. To overcome these existing barriers, we have developed a novel rapid, high throughput molecular diagnostic platform. Species-specific primers and probes were generated and used for the 8 most common gastrointestinal parasite pathogens -- *Ascaris lumbricoides, Necator americanus, Ancylostoma duodenale, Giardia lamblia, Cryptosporidium parvum, Entamoeba histolytica, Trichuris trichiuria* and *Strongyloides stercoralis* – in a standardized and quantitative multi-parallel fast chemistry-based real time PCR (qPCR) high throughput assay. This was then used to analyze stool samples collected from 540 children in rural Ecuador and compared to standard stool microscopy. The qPCR improved the sensitivity for *Ascaris* by 21.9% and had 97.8% specificity and 96.8% negative predictive value. Moreover there was a direct relationship between the egg counts by stool examination with nanograms of *Ascaris* DNA (spearman r: 0.69, p=0.0001). qPCR improved the sensitivity for *Giardia* by 81.7% (126/400 for qPCR vs. 23/400 for stool examination p=0.001) In addition, the qPCR assays were able to detect infections for *Ancylostoma, Cryptosporidium, Strongyloides* not seen by microscopy. It further provided methods to distinguish the pathogenic *Entamoeba histolytica* from the non-pathogenic *E. dispar.* Finally, by using a novel DNA extraction protocol, we were able to detect *Trichuris,* with an improved sensitivity of 75% and a direct correlation to DNA (spearman r: 0.74, p=0.0001). Thus, we have developed a high throughput, rapid, operator-independent molecular-based system to improve the diagnostic accuracy of stool based parasite pathogen detection. This approach has been field tested in rural Ecuador and will be useful to refine treatment options for affected populations and ultimately lead to better health outcomes in resource-limited settings.

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EVALUATION OF ALTERNATIVE DIAGNOSTIC METHODS FOR SCABIES AND STRONGYLOIDIASIS

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Scabies and strongyloidiasis are neglected tropical diseases which are responsible for considerable morbidity worldwide, primarily in disadvantaged and overcrowded communities. Scabies is a highly infectious skin disease caused by infestation of the skin with the ectoparasitic mite *Sarcoptes scabiei*. Up to 300 million people worldwide estimated to be afflicted at any time. In addition the scabies mite causes mange in many livestock, companion and wild animal populations. Strongyloidiasis is caused by infection with the parasitic nematode *Strongyloides stercoralis* and is associated with high mortality in immunosuppressed individuals. Each of these diseases is endemic in many remote Indigenous communities in the tropical north of Australia. A mass drug administration trial for the control of scabies and strongyloidiasis was conducted in a remote Indigenous community in the Northern Territory of Australia. Diagnosis of scabies was made by a clinical evaluation of participant's skin. Diagnosis of strongyloides in children was made by direct microscopic examination of faecal samples followed by agar plate culture and formalin sedimentation. This study aimed to investigate: i) methods for the diagnosis of strongyloidiasis which are more logistically appropriate for use in remote and resource poor settings and; ii) improved methods of diagnosis of scabies compared with clinical diagnosis which lacks sensitivity. PCR based methods were trialled for the identification of *S. scabiei* in skin samples and of *S. stercoralis* in faecal specimens. *S. scabiei* gene specific PCR was more sensitive than microscopic examination for the detection of *S. scabiei* in skin samples and is highly specific. PCR targeting the 18S rRNA gene was at least as sensitive as microscopy and agar plate culture for the detection of *S. stercoralis*. Thus PCR may be a useful alternative to the clinical diagnosis of scabies and when parasitological analysis of fresh faecal samples is the field is logistically difficult.

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ASYMMETRICAL ISOTHERMAL AMPLIFICATION METHOD FOR GENOTYPING MUTATIONS, IN HUMAN SOIL-TRANSMITTED HELMINTHS, THAT HAVE BEEN ASSOCIATED WITH BENZIMIDAZOLE RESISTANCE

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Soil-transmitted helminths (STHs), *Ascaris lumbricoides* and *Necator americanus,* are gastrointestinal nematodes causing human morbidity in tropical areas of the world. Benzimidazole (BZ) drugs, albendazole and mebendazole have been used extensively for large-scale treatment of STHs. A growing concern is that extensive use of anthelmintics to control human parasites is likely to exert selection on parasite populations as has occurred in gastrointestinal nematodes of livestock. The egg reduction rate has been used to monitor drug efficacy and to detect the development of resistance in the field. This assay is very insensitive for the detection of low levels of drug resistance. Previous molecular assays for putative resistance mutations have been based mainly on sequencing. However, sequencing is a time consuming and complicated procedure, not suitable for routine clinical use or for resource constrained situations.Therefore, development of simple, rapid and cost-effective molecular tools for detecting BZ resistance, that could be adaptable to field conditions, would be very helpful for sustainable control of STHs. We developed a novel genotyping assay based on the Smart Amplification Process (SmartAmp2) to detect mutations of the β-tubulin isotype 1 gene associated with BZ resistance under isothermal conditions without PCR amplification. This isothermal method uses asymmetrical primers and the mismatch-binding protein MutS to prevent mismatch amplification giving high specificity. For experimental development, real-time PCR monitoring of the amplification was achieved within 40-60 min with suppression of the mismatch amplification. Wild-type and mutant plasmids were employed to develop and optimize the assay. The assays were applied to analyze fecal samples of eggs and larvae using full-match and mismatched primer sets. A SmartAmp2 assay was developed for genotyping the mutations in the β-tubulin gene in *A. lumbricoides* and *N. americanus* and the reliability of the method was validated using the conventional PCR method. Work is being conducted to use end point detection system to enable this technique to detect mutations associated with BZ resistance in the field.

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THE EFFECTS OF HOST DIETARY FACTORS ON CURE RATE AND FECAL EGG COUNT REDUCTION FOLLOWING SINGLE DOSE ALBENDAZOLE (400 MG) AMONG SCHOOL-AGE CHILDREN INFECTED WITH HOOKWORM IN THE KINTAMPO NORTH DISTRICT OF GHANA

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Ghanaian school-age children (n=141) from five communities previously identified as having high prevalence of hookworm infection and reduced cure rates were enrolled in a cross-sectional study investigating host predictors of treatment response. Household surveys were used to collect dietary patterns, socioeconomic and demographic information, and other health indicators. Infection status and dietary diversity data were assessed to identify modifiable host factors that might affect treatment response. Prevalence of hookworm infection was 56% (79/141). Those positive for hookworm were treated with single-dose albendazole (400 mg). Immediately prior to treatment, a brief screening questionnaire was administered to identify recent dietary patterns and diarrhea. Consumption of more diverse food groups in the twenty-four hours prior to treatment, as indicated by dietary diversity scores above the median, was associated with better rates of hookworm clearance after treatment (48.2% cure rate) compared to those consuming fewer food groups (12.5% cure rate) ($\chi 2$; $p < 0.01$). Dietary protein showed a stronger effect than dietary diversity: all children below the population median of protein food groups remained infected with hookworm following treatment, and children above the median had a 42% cure rate. Individuals who had not eaten six hours prior to treatment had better drug responses and were 5.0 times more likely to experience higher egg reduction rates than those who ate in the six hours prior to treatment ($p < 0.05$). Dietary diversity also significantly impacted fecal egg reduction rates in treated individuals. Those with higher dietary diversity were 3.1 times more likely to be in the highest category of egg reduction rate than those with lower dietary diversity ($p < 0.05$). These findings provide new data on the relevance of dietary patterns in affecting cure rates and egg reduction rates following single-dose albendazole therapy. Further work is needed to determine whether these factors could be used to improve operational effectiveness of mass drug administration.

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HELMINTH INDUCED ALTERNATIVE MACROPHAGE ACTIVATION AND MYCOBACTERIAL (BCG) INFECTIONS

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Filarial and other tissue invasive helminth infections have been associated with alternative activation of macrophages (AAM), a process felt to reflect the influence of Type 2 responses (IL-4/IL-13) on macrophage differentiation. In contrast, *Mycobacterium tuberculosis* (Mtb) infection requires a strong Type 1 (IL-12/IFN-γ) response to control the infection in the macrophage. The intersection of these two highly prevalent infections was studied initially in C57BL/6 mice injected intravenously with *B. malayi* microfilariae (mf) prior to an aerosolized infection with Mtb. We found by RT-PCR that filarial infection was associated with a two fold induction of YM1 and CD206 in the lungs of microfilaremic mice with an almost 100 fold decrease in the expression of iNOS, findings consistent with the generation of AAM in the lungs. This AAM phenotype was overcome in the context of Mtb co-infection. Since the macrophage is thought to be critical to innate control of mycobacterial infection, we sought to address the role of AAM in the control of mycobacterial infection in the context of filarial/mycobacterial co-infection using an *in vitro* model system. Purified human monocytes were used to generate macrophages (M-CSF for 7 days) after which both classically activated ([CAM] with LPS and interferon gamma (IFN-)) and AAM (with interleukin-4 [IL-4]) were generated. After 48 hours of polarization, infection with mycobacteriae (BCG) at an MOI of 5 was performed and 24 later responses were contrasted between CAMs and AAMs. With the exception of CCL13 and CCL22 production in the AAMs, there were no differences between the CAM and AAM in the spontaneous mRNA expression of chemokines such as CCL-17, CCL-18, IL-18,PDCDL1G,CLEC10A, CADH1,CD274 or IL-12p40,TNF,IL-6,IL-1a,IL-1b,IL-10 protein (by Luminex™). In contrast, following infection with BCG, AAM had significantly increased production of IL-10 (median=1162 pg/ml vs. 504.77 pg/ml, p= 0.03) and decreased production of IL-1b (median=384.2 pg/ml vs. 1563.09 pg/ml, p=0.01) and IL-6 (median=3296.8 pg/ml vs 11357.16 pg/ml, p=0.03) in the 8 monocyte donors tested. CCL13 expression, in contrast was significantly downregulated following BCG infection in the AAMs (~10 fold decrease in expression, p=. 007) compared to CAMs. These data suggest that an altered response to mycobacterial infection is exhibited by AAMs compared to CAMs that may alter the outcome of these infections.

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IMMUNOLOGICAL BASIS OF SUPERIOR PROTECTION FOLLOWING INFECTION-TREATMENT IMMUNIZATION COMPARED TO IRRADIATED SPOROZOITE IMMUNIZATION

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Immunization of both humans and rodents with either radiation-attenuated *Plasmodium* sporozoites (RAS) or infection-treatment sporozoite immunization (ITI) elicits sterilizing anti-malarial immunity against subsequent sporozoite challenge. In rodent models, protection following RAS or ITI requires the induction and activity of parasite-specific CD8 T cells. Here we show that both ITI and RAS vaccinations elicit parasite-specific CD8 T cells exhibiting equivalent expression of molecules associated with T cell activation, inhibition, migration, and survival. However, compared to RAS, ITI requires fewer immunizing sporozoites to elicit sterilizing immunity. Moreover, single dose ITI induces larger effector and memory CD8 T cell responses leading to complete protection upon sporozoite challenge. Importantly, ITI-induced CD8 T cells exhibit specificity for a broader profile of parasite antigens compared to RAS. Consistent with this, we find that sterilizing protection following ITI is associated with a short duration (4-6 days) and low magnitude (up to 4-7%) level of blood-stage breakthrough parasitemia shortly following chloroquine cessation. Further, ITI mice resist challenge with blood-stage parasites. Collectively, our data show the cellular basis for potent cross-stage immunity elicited by ITI depends on the induction of CD8 T cell responses and exposure to blood-stage parasites.

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DEVELOPMENT OF *TRYPANOSOMA CRUZI* GENETICALLY ATTENUATED KNOCKOUT LINES WITH POTENTIAL USE AS TRANSMISSION BLOCKING VACCINES

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Chagas disease, caused by the protozoan *Trypanosoma cruzi*, is the most important parasitic burden in Latin America. There are no effective vaccines to prevent this infection. In endemic areas, dogs are important sources of infection for the insect vector and therefore represent a critical control point for *T. cruzi* transmission. A transmission-blocking vaccine for dogs would greatly reduce the prevalence of *T. cruzi* infection in the canine and consequently, in the human population. Live attenuated parasites can be used as experimental vaccines. In this work we report on the generation of *T. cruzi* attenuated lines (KO5: Serine/threonine protein phosphatase like-protein; KO10: Hypothetical protein; KO121: Protein kinase and ECH: Enoyl-CoA hydratase/isomerase family protein) by disruption of genes, whose products are predicted to be critical for parasite replication in mammals. We evaluated whether C57BL/6 mice immunized with these attenuated parasites would develop protective immune responses that would prevent the establishment of vector transmittable infection upon rechallenge with *T. cruzi*. Mice immunized with any of the attenuated lines elicited strong *T. cruzi* -specific CD8+ T cells responses. However, the frequencies of *T. cruzi* -specific CD8+ T cells in mice immunized with the KO10 line decreased to undetectable levels in the blood after ~70 days post immunization (dpi). At 300 dpi, parasite-specific CD8+ T cells from mice immunized with KO5 and ECH showed relatively high expression of the central memory marker CD127 and low expression of recent activation marker KLRG1 compared with their wild type and KO121 counterparts. The magnitude and the phenotype of *T. cruzi* -specific CD8+ T cells suggest that these lines could be ideal for a transmission blocking vaccine for dogs. Current studies are focused on determining parasite persistence and on whether mice immunized with these attenuated lines and rechallenged with a virulent *T. cruzi* strain will not develop blood parasite levels sufficiently high to infect the insect vectors and therefore block the transmission of the infection.

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IDENTIFICATION OF NOVEL HIGHLY PROTECTIVE PRE-ERYTHROCYTIC ANTIGENS FOR MALARIA VACCINE DEVELOPMENT

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Malaria is the most devastating parasitic disease affecting humans. There is no licensed malaria vaccine. Efforts to develop an effective malaria

vaccine have been limited by the small number of known antigens, which represent < 0.5% of the 5300 encoded proteins in the *Plasmodium falciparum* genome. Most vaccines under clinical evaluation today contain only one antigen and are only partially protective. The most advanced candidate, RTS,S, provides about 50% protection. Thus, there is a great need for an effective malaria vaccine that could provide robust protection and contribute to the control and eventually eradication of malaria. Our vaccine rationale is based on the fact that immunization with radiation-attenuated sporozoites (RAS) provides high level (>90%) protection against sporozoite challenge in both mice and humans, and this protection is dependent on the induction of $CD8^+$ T cells targeting multiple antigens expressed in the pre-erythrocytic stages of the parasite life cycle. The circumsporozoite protein (CSP) is one of the targets of protective T cell responses induced by RAS immunization. However, other unknown antigens clearly contribute to protection. The goal of our research was to identify novel antigens that are the targets of protective T cell responses in mice immunized with protective regimens of RAS. We have identified several new and highly protective pre-erythrocytic stage antigens using a novel high-throughput genomics screening approach. Our antigen discovery system utilizes an array of adenovirus vectors carrying 300 highly expressed *P. yoelii* pre-erythrocytic genes with identifiable *P. falciparum* orthologues. In the antigen discovery screen, antigen presenting cells were infected with individual adenovectors from the array and then mixed with splenocytes from mice immunized with protective regimens of RAS. We prioritized antigens based on the frequency of $CD8^+$ T cell recall responses to each of the 300 antigens. We selected 20 antigens that recalled the most robust T cell responses and tested their capacity to protect mice from a *P. yoelii* sporozoite challenge. Outbred CD1 mice were immunized with a DNA prime - Ad boost regimen and sterile protection was measured following sporozoite challenge. Several of the prioritized antigens induced higher levels of protection than CSP. The *P. falciparum* orthologues of these antigens are being considered for advancement to clinical development.

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THE TRANSCRIPTION FACTOR T-BET REGULATES PARASITEMIA AND PROMOTES PATHOGENESIS DURING MURINE MALARIA

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We investigated the role of the transcription factor T-bet (the master regulator of Th1) cells during murine malaria infections. Using mice deficient for T-bet, we report that T-bet interferes with the formation of a protective immune response during *Plasmodium yoelii* 17XNL murine malaria. On day 12 post-infection, T-bet deficient mice (5.7 ± 1.6%) had 2.9 fold lower parasitemia (p=0.0013) than wildtype controls (16.8 ± 1.7%). Although T-bet deficient mice had diminished levels of total IgG antibody, these mice had significantly higher levels of IgG1 (81,920 ± 12,540) compared to wildtype controls (16,640 ± 3840) indicating that this antibody isotype may be important for malaria protective immunity (p=0.0011, Student T test). In the *Plasmodium berghei* ANKA murine model of experimental cerebral malaria (ECM), we demonstrate that while T-bet regulates parasite burden, it also promotes the pathogenesis of ECM possibly by impeding the formation of an anti-inflammatory Th2 immune response. T-bet deficient mice had higher parasitemia than wildtype controls during the ECM phase of disease (17.7 ± 3.1% verses 10.9 ± 1.5%). In addition, while 100% (10/10) of wildtype mice developed ECM by day 9 post-infection, only 30% (3/10) of T-bet deficient mice succumbed to disease during the cerebral phase of infection (p=0.000029, Log rank). Resistance to ECM in T-bet deficient mice was associated with a Th2 immune response characterized by enhanced production of GATA-3^+ $CD4^+$ T cells and elevated levels of the eotaxin, MCP-1, and G-CSF cytokines. Our results suggest that in the mouse models studied, Th1-type immune responses render deleterious outcomes as they interfere with the acquisition of immunity and mediate the pathogenic features of cerebral malaria.

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ANTIBODY DYNAMICS AFTER ACUTE MALARIA INFECTION IN CHILDREN

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Naturally acquired antibodies directed against *Plasmodium falciparum* are acquired slowly with repeated infections and protect against malaria disease. However children have been shown to generate short-lived anti-malaria antibodies. While this may be due in part to a less mature immune system, malaria infection may also exert immuno-modulatory effect. Our goal was to examine children's antibody signature after acute clinical malaria in order to categorize stability of responses. Children (n=89, mean age 25 months (range 1 - 66 mo)) were recruited from Chulaimbo sub-District Hospital in western Kenya upon presentation with a febrile illness. Participants diagnosed with acute malaria provided a venous blood sample, were treated with 6-doses of CoArtem™ (Artemether/Lumefantrin), and examined 4 weeks later (recovery) when another blood sample was drawn. Plasma samples were examined for the prevalence and magnitude of anti-malaria antibodies by a) luminex multiplex serology to 13 malaria antigens and b) functional antibody-mediated growth inhibition of cultured parasites. We found that total IgG to $MSP\text{-}1_{42}$ (3D7, FVO, and FUP) and PfCelTOS declined from acute to recovery time points. No change was detected in IgG levels to AMA1 (3D7 or FVO), PfCSP, EBA140, EBA175, EBA181, and SERA5 (two variants). In contrast, functional sialic-dependent pathway inhibitory antibodies and global growth inhibition antibodies against W2mef were boosted after an episode of symptomatic malaria. $MSP1_{19}$ invasion inhibitory antibodies and global growth inhibition antibodies against PfD10 remained unchanged after infection. In addition, we found that IgG to measles decreased after an episode of malaria. Total IgG did not however change significantly between the time points. Thus, acute clinical malaria infections differentially influence the maintenance of anti-malaria antibodies as well as have potential detrimental consequences for immunity against measles.

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RHOPTRY NECK PROTEIN 2 IS PRODUCED IN OOCYST-DERIVED SPOROZOITES AND REQUIRED FOR SALIVARY GLAND INVASION

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During apicomplexan parasite invasion, it is revealed that tight junction is formed between parasite and target cells, which confers parasite move inside cells. Recently in *Plasmodium* merozoite, it was reported that rhoptry neck protein 2 (RON2) is secreted to the tight junction and RON2 and AMA1 interaction is important during erythrocyte invasion. However, ron2 disrupted transgenic parasite has never been successfully generated presumably because RON2 is essential for proliferation in the blood stage. Here, using *Plasmodium berghei*, we showed that RON2 is produced also in sporozoites and localized to rhoptry. Interestingly, ron2 transcription is restricted to midgut sporozoites, however RON2 protein can be detected through sporozoite migration from midguts to salivary glands. To elucidate sporozoite RON2 function, we generated sporozoite stage specific RON2 silencing transgenic parasites by swapping ron2 promoter to merozoite specific promoter. The number of sporozoites collected from

salivary glands was greatly reduced by ron2 silencing, despite sporogony, sporozoite release into hemocoel and their motility were normal. These results showed that RON2 is required for salivary gland invasion. This is the first genetical approach to show that RON2 has an important role in target cell invasion.

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IDENTIFICATION AND CHARACTERIZATION OF A *PLASMODIUM FALCIPARUM* ORTHOLOGUE OF THE YEAST UBIQUINONE-BINDING PROTEIN, COQ10P

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Coenzyme Q (CoQ, ubiquinone) is a central electron carrier in mitochondrial respiration. CoQ is synthesized through multiple steps involving a number of different proteins. The prevailing view that the CoQ used in respiration exists as a free pool that diffuses throughout the mitochondrial inner membrane bilayer has recently been challenged. In the yeast *Saccharomyces cerevisiae*, deletion of the gene encoding Coq10p results in respiration deficiency without altering total size of the available CoQ pool, suggesting that the Coq10p is critical for the delivery of CoQ to the site(s) of respiration. The precise mechanism by which this is achieved remains unknown at present. Because mitochondrial respiration is a validated target for antimalarial drugs such as atovaquone, we are interested in examining its regulation in malaria parasites. We have identified an orthologue of Coq10p, PfCoq10, in *P. falciparum*, the most virulent species of malaria parasite, and demonstrated that a GFP-tagged version of PfCoq10 localized to the parasite mitochondrion. Expression of PfCoq10 in the *S. cerevisiae coq10* deletion strain restored the capability of the yeast to grow on respiratory substrates, suggesting a remarkable functional conservation of this protein over a vast evolutionary distance, and despite a relatively low level of amino acid sequence identity. We are currently assessing effects of PfCoq10 overexpression on the atovaquone sensitivity of *P. falciparum*. We are also examining the possibility of altered response to atovaquone in yeast mitochondria expressing the parasite Coq10. These studies may provide insights into respiration regulation in general, as well as in malaria parasites.

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ROLE OF PFRAD54 AND REPLICATION PROTEIN A IN RAD51-MEDIATED DNA STRAND EXCHANGE AND REPAIR OF DNA DAMAGE INDUCED BY MMS IN *PLASMODIUM FALCIPARUM*

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Exploiting the recombination machinery and its molecular characterization in the malaria parasite would provide mechanistic understanding of recombinational rearrangements leading to immune evasion via antigenic switching, a major impediment in developing an effective vaccine against these protozoan parasites. Bacterial RecA protein and its eukaryotic homologue Rad51 play a central role in homologous DNA strand exchange reaction during recombination and DNA repair. Previously, our lab has shown that PfRad51, the *Plasmodium falciparum* homologue of Rad51, exhibited ATPase activity and promoted DNA strand exchange *in vitro*, as reported previously. Here, we evaluated the catalytic functions of PfRad51 in the presence of putative interacting partners, especially *P. falciparum* homologues of Rad54 and Replication protein-A (RPA). PfRad54 accelerated PfRad51 mediated pairing between ssDNA and its homologous linear dsDNA in the presence of 0.5mM CaCl2. We also present evidence that recombinant PfRPA1L protein serves the function of bacterial homologue SSB in initiating homologous pairing and strand exchange activity but its function was negatively regulated in a dose-dependent manner by PfRPA1S, another RPA homologue in *P. falciparum*. We also present *in vivo* evidence through comet assays for methyl methanesulfonate (MMS)- induced DNA damage in malaria parasites and accompanying upregulation of PfRad51, PfRad54, PfRPA1L and PfRPA1S at the level of transcript and protein. This study provides new insights into the role of putative Rad51-interacting proteins involved in homologous recombination and emphasizes physiological role of DNA damage repair during the growth of parasites. We are now characterizing the recombiantion macromolecular complex which is likely to be important in DNA damage and repair and validating molecular interactions between PfRad51 and its putative interacting partners. Besides understanding molecular machinery involved in DNA repair and recombination, we wish to extend our studies to understand the biochemical and genetic basis of gene rearrangements at the var gene locus associated with phenomenon like antigenic variation.

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A SINGLE NUCLEOTIDE POLYMORPHISM IN THE PROMOTER OF STROMAL CELL-DERIVED FACTOR (SDF)-1A (C-1002T) IS ASSOCIATED WITH PROTECTION AGAINST *PLASMODIUM FALCIPARUM* INFECTION IN KENYAN CHILDREN

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Stromal cell-derived factor (SDF)-1α (CXCL12) is a pleiotropic chemokine with diverse functions including induction of anti-pathogen immunity and inhibition of erythropoiesis. In murine malaria, increased expression of SDF-1α promotes control of parasitemia. Although several studies indicate that *SDF1A* genetic variation regulates outcomes in the context of HIV-1 infection, hematopoiesis, and cancer, the role of genetic variability in *SDF1A* in *Plasmodium falciparum* infections has not been explored. The effect of *SDF1A* (C-1002T, rs2839686) variation was therefore, investigated in Kenyan children (2.0-38.0mos., n=873) residing in a holoendemic *P. falciparum* transmission region of western Kenya. Children were stratified into aparasitemic (n=212) and parasitemic (n=661) groups with parasitemic children being further categorized into SMA (hemoglobin, Hb<5.0g/dL; n=236) vs. non-SMA (Hb≥5.0g/dL; n=425), high-density parasitemia (HDP; ≥10,000 parasites/μL; n=477) vs. low-density parasitemia (LDP; <10,000 parasites/μL; n=184), and reticulocyte production index (RPI<2.0) vs. (RPI≥2.0). Multivariate logistic regression modeling controlling for age, gender, bacteremia, glucose-6-phosphate dehydrogenase, alpha-thalasemia, and sickle cell and HIV-1 status did not show any significant associations between carriage of C-1002T genotypes and SMA, RPI<2.0, and HDP. However, carriage of the CC genotype was associated with protection against the acquisition of *P. falciparum* infection compared to the TT genotype (Odds ratio, OR, 0.311; 95% CI, 0.115-0.842; *P*=0.022). These results demonstrate that although variation at 1002 in the *SDF1A* promoter appears to protect against acquisition of *P. falciparum* infection, this variant may not affect malaria disease outcomes once an individual becomes infected.

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GENOMIC DIVERSITY AND EVOLUTIONARY HISTORY OF *PLASMODIUM VIVAX*

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Most studies of genetic diversity in *Plasmodium vivax* have focused on microsatellites or selected loci and do not provide a genome-wide perspective. We have sequenced the genomes of ten *P. vivax* field isolates

collected across the world, and three monkey-adapted strains. We sequenced each sample on one lane of an Illumina HiSeq 2000 to obtain 70-400X coverage and have >93% of the reference genome covered by >20 reads. We conservatively identified >85,000 SNPs across the genome as well as several sequence rearrangements. The genetic diversity is significantly higher in intergenic regions than in coding regions (6.84 vs. 3.24 SNPs/kb) with intronic sequences harboring an intermediate level (4.63 SNPs/kb). We also observed 1.5-fold more non-synonymous than synonymous SNPs while, based on the genome composition, we would expect 4-fold more nsSNPs. These observations suggest that evolution of most *P. vivax* protein-coding sequences is driven by purifying selection. To identify genes evolving under positive selection, we compared the rates of non-synonymous (PN) and synonymous substitutions (PS) for all protein-coding sequences. Two red blood cell binding protein genes, the Duffy binding protein and the reticulocyte binding protein 2 like, were among the ten genes with the highest PN/PS ratio (2.14 and 1.89 respectively), suggesting that they are evolving under strong positive selection. The reticulocyte binding protein genes 1 and 2 also displayed PN/PS ratios consistent with positive selection. The PN/PS ratio for other red blood cell binding proteins, such as the apical merozoite antigen 1 or the rhoptery neck protein 1 and 2 genes, was more consistent with negative selection. Our genome-wide analyses of selective constrains in *P. vivax* suggest that natural selection is actively changing the amino acid sequences of proteins involved in early stages of erythrocyte invasion (merozoite attachment to red blood cells and reorientation), while proteins involved in latter stages (junction formation and invasion) are more conserved.

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GENOME-WIDE ANALYSIS OF NATURAL SELECTION IN *PLASMODIUM FALCIPARUM* POPULATIONS IN WEST AFRICA

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When *Plasmodium falciparum* competes with other genotypes in same host or when under drug and host immunity pressure there is selection on the genome which leaves marks that can be identified by population genetic methods. Selective pressure will vary in different geographical locations and the varying marks detected in this case will correspond to genes involved in local adaptation. To explore this, we first analysed population structure by genotyping ten polymorphic *P. falciparum* microsatellite loci in 268 infections from eight locations in four West African countries (Republic of Guinea, Guinea Bissau, The Gambia and Senegal), spanning a highly endemic forested region in the south to a low endemic Sahelian region in the north. This showed that each location had similar levels of genotypic diversity, although there were many more mixed parasite genotype infections in the south. Genetic differentiation between populations was low and an overall test for isolation by distance was not significant. Given the high levels of recombination and minimal reproductive isolation of parasite populations in West Africa, differential signatures of selection in particular populations should be detectable against a background of neutral genomic variation that is more spatially homogeneous. To address this, we undertook a population genomic analysis of parasites at a highly endemic site in the Republic of Guinea, with whole genome sequencing using Illumina 76 base paired-end reads mapped to the reference genome of *P. falciparum*. Genome-wide analysis of SNPs in 100 *P. falciparum* clinical isolates from this population was used to identify genes under natural selection, and we compare the findings with those of a lower endemic site in The Gambia.

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TRACKING CHANGING POPULATION DYNAMICS OF *PLASMODIUM FALCIPARUM* INFECTIONS REVEALS EVIDENCE OF CLONAL INFECTIONS FOLLOWING INTRODUCTION OF TRANSMISSION-REDUCING INTERVENTIONS IN THIÉS, SENEGAL

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As global health efforts to control and eliminate malaria gain momentum, genetic tools can aid in monitoring malaria transmission dynamics, detecting emergence of drug resistant parasites, and identifying sources of new malaria infections. Using genotyping tools, we identified a large number of clonal infections among patients with mild uncomplicated malaria seeking treatment at a clinic in Thiès, Senegal. While it is not unexpected to find limited instances of the same parasite within households or related individuals, the occurrence of identical parasites has been increasing since 2006 (P-value = 0.006), with more than 25% of the parasites collected from geographically and temporally distinct patients sharing an identical molecular barcode in 2008 and 2011. We validated the sensitivity of the molecular barcode to detect parasites that are nearly genetically identical by sequencing and hybridization to Affymetrix SNP genotyping arrays. These clonal findings are coincident with increased deployment of malaria control interventions and decreased malaria deaths in Senegal from 2006 to 2011. In addition, parasite types identified in one transmission season were present in subsequent seasons, providing evidence that specific parasite lineages persist across years. Further, the appearance and rise of clonal parasites corresponded with a substantial decrease in the effective parasite population size calculated by several methods from a high of over 106 parasites in 2006 to less than 40 by 2011. These data provide the first evidence of a strong temporal correlation between the appearance of clonal parasite types and increased malaria control interventions_including distribution of insecticide treated nets; use of rapid diagnostic tests for malaria detection; and deployment of artemisinin combination therapy approaches. Our results demonstrate that population genetic based tools can be used as a diagnostic tool to detect changes in malaria intensity and evaluate the effectiveness of local control and elimination strategies to best inform a global malaria eradication campaign.

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WOLBACHIA-BASED INTERVENTIONS FOR MALARIA VECTOR CONTROL

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Malaria, transmitted by *Anopheles* mosquitoes, remains a major global public health problem. Novel vector control interventions are urgently needed to address vector species that are not effectively targeted by current tools. *Wolbachia* is a maternally transmitted symbiotic bacterium that can not only spread within mosquito populations through its unique ability to manipulate mosquito reproduction but can also induce resistance to human pathogens in mosquito vectors. We have now successfully established a stable *Wolbachia* infection in a major Asian malaria vector, *Anopheles stephensi*. This vector-resident *Wolbachia* has a 100% maternal transmission rate and induces nearly complete cytoplasmic incompatibility. Laboratory population replacement experiment shows *Wolbachia* could reach 100% infection frequency within seven generations after release.

These results open the possibility to use *Wolbachia* as a novel intervention tool for malaria control, as successfully demonstrated in *Aedes* mosquitoes.

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WOLBACHIA USES HOST MICRORNAS TO FACILITATE COLONIZATION OF THE DENGUE VECTOR *AEDES AEGYPTI*

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Wolbachia are maternally inherited, gram-negative endosymbiotic bacteria, which are commonly found in invertebrates. It is estimated that more than 65% of all insect species are infected with *Wolbachia*. They are best known for manipulating the host reproductive systems through a variety of strategies such as cytoplasmic incompatibility, male-killing, feminization and parthenogenesis. The *Wolbachia* strain *w*MelPop has been shown to be able to modulate lifespan of host insects and interfere with development of human pathogens in mosquito vectors. However, very little is known about the molecular basis of the interactions. Understanding the mechanisms involved in the interactions may facilitate devising novel approaches to inhibit/limit transmission of mosquito-borne pathogens. Using microarrays and deep sequencing, we have shown that the endosymbiont manipulates the host mosquito's (*Aedes aegypti*) microRNA (miRNA) profile to facilitate its replication/maintenance in the host cells. In addition, alterations in miRNA and cellular proteins involved in miRNA trafficking are observed in *Wolbachia*-infected mosquitoes. Results pertaining identification and functional characterization of a number of miRNAs manipulated by *Wolbachia* that play significant roles in host colonization and biology will be discussed.

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IVERMECTIN INHIBITS AND DELAYS THE DEVELOPMENT OF *PLASMODIUM FALCIPARUM* IN *ANOPHELES GAMBIAE*

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Multiple ivermectin mass drug administration (MDA) to humans in southeastern Senegal reduced both the survivorship of *Anopheles gambiae* s.s. and the proportion of *Plasmodium falciparum*-infectious *An. gambiae*. Ivermectin has been shown to impact several factors of vectorial capacity including: the time between blood meals, the daily probability of mosquito survivorship, and by extension the vector-to-host ratio. Here we investigated whether ivermectin alters two other factors of vectorial capacity, the proportion of *An. gambiae* that become infective and the time for parasite development. Indeed, ivermectin inhibits and delays the development of *P. falciparum* (NF 54) in *An. gambiae* (G3). Ivermectin was fed to *An. gambiae* at the lethal concentration that kills 25 percent of mosquitoes (LC_{25}). Mosquitoes were dissected on days post infection (DPI) 7, 12 and 14. When ivermectin and *P. falciparum* were co-ingested at the same time the proportion of vectors with oocysts (LC_{25} DPI 7, P=0.0013) and sporozoites (LC_{25} DPI 12, P=0.0286, LC_{25} DPI 14, P=0.00133) was reduced. Development of *P. falciparum* in the vector was delayed as evidenced by analysis of rates of change in the proportion of control mosquitoes between DPI 12 = 0.5648 and DPI 14 = 0.7720 (P=0.0651) and treatment mosquitoes between DPI 12 = 0.1933 and DPI 14 = 0.4342 (P=0.0486). Development in the vector was inhibited when ivermectin was ingested six DPI (LC_{25} DPI 14, P=0.0317), but not three DPI (LC_{25} DPI 14, P=0.7193). This suggests that ivermectin may inhibit development of late stage but not early stage oocysts. This work demonstrates that ivermectin inhibits oocyst development and is sporontocidal, although the mechanism of action has not been determined yet. Ivermectin MDA is the only malaria transmission control tool currently available that can impact all factors of vectorial capacity. Future work will utilize field isolates of *P. vivax* and *P. falciparum* to determine the impact of ivermectin on *Plasmodium* development in Thai malaria vectors.

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THE EXPRESSION OF OLFACTION GENES IN *ANOPHELES GAMBIAE* IN RELATION TO HUMAN HOST PREFERENCE

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Females of *Anopheles gambiae*, the main vector of human malaria in Sub-Saharan Africa, have a strong attraction to odors of humans, their primary host. Several gene families involved in the olfaction system; the olfactory receptors (ORs), Ionotropic receptors (IRs), and odorant binding proteins (OBPs), interact directly with odorants. As such they are prime candidates for contributing to the strong human host preference of *An. gambiae*. To understand the molecular basis of anthropophily in *An. gambiae*, we are examining the differential expression of olfaction genes in *An. gambiae* and *An. quadriannulatus*, a zoophilic species within the *An. gambiae* species complex. Our expectation is that olfaction genes important in human host preference will be expressed at higher relative levels in *An. gambiae* compared to *An. quadriannulatus*. Using Illumina high-throughput sequencing, we have generated transcriptome data from the heads (including antennae and maxillary palps) of backcrosses between the two species. Backcross female host preference was assessed using an olfactometer. Two RNA-seq libraries consist of backcross females who prefer human hosts, and two RNA-seq libraries consist of backcross females who prefer cow hosts. We identified 31 olfaction genes that are expressed more than two-fold in the human vs the cow preferring pool: 20 ORs, 10 OBPs, and 1 IR. If these up-regulated olfaction genes are involved human host preference, we also expect them to be expressed at higher levels in the antennae/palps of *An. gambiae* females compared to males or *An. quadriannulatus*. We have therefore analyzed the transcriptomes of both the antennae and maxillary palps from the females and males from both species. This study allows us to identify candidate genes responsible for the adaptation of *An. gambiae* to human hosts and that may provide promising targets for designing repellents/attractants or transgenic approaches for controlling *An. gambiae* populations.

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EXPRESSION OF THE OLFACTION GENE REPERTOIRE IN *AEDES AEGYPTI* FOLLOWING BLOOD FEEDING

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Mosquito host preference is a complex trait that is closely tied to disease transmission and presumably involves the precise regulation of numerous of olfaction related genes. The yellow fever mosquito *Aedes aegypti* displays considerable behavioral and physiological changes after acquiring a blood meal and females suppress host seeking behavior following a blood meal. Numerous genes belonging to three families; the olfactory receptors (ORs), the ionotropic receptors (IRs), odorant binding proteins (OBPs) are potentially involved in host seeking behavior and are expressed in the antennae. We expect unfed female *Ae. aegypti* to express genes needed to locate human hosts, whereas those that are blood fed do not, or do so at a reduced level. RNA-seq was used to quantify changes in the expression of protein coding genes in the main olfaction organs of *Ae. aegypti*, the antennae. Four days old females were blood fed and antennae were collected prior to and 3, 24, 48 and 72h after feeding. Differential expression analyses were performed using the unfed females as a reference. The total number of genes differentially expressed were 63, 111, 107, and 94 for 3, 24, 48 and 72h, respectively. Three gene families were analyzed in detail: Olfaction Receptors (ORs), Ionotropic Receptors (IRs), and Odorant Binding Proteins (OBPs). Most (62.5%) of the OBPs are highly expressed (FPKM>1000), whereas most IRs (65%) are not expressed in the antenna of females. ORs tend be expressed at intermediate levels; 55.1% of annotated ORs with FPKM from 10 to 100. Most of the ORs are down-regulated after feeding, but typically the

changes in expression are small. The expression profile of OBPs and IRs does not change considerably after feeding compared to ORs. Genes of particular interest are OR46, OR99, and one IR (AAEL005039), that are expressed in unfed females only. Genes differentially expressed or those only expressed before or after feeding, are candidate host seeking genes.

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IGG RESPONSES TO *ANOPHELES GAMBIAE* SALIVARY ANTIGEN GSG6 DETECT VARIATION IN EXPOSURE TO MALARIA VECTORS AND PREDICT DISEASE RISK

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Assessment of exposure to malaria vectors is important to our understanding of spatial and temporal variations in disease transmission and facilitates the targeting and evaluation of control efforts. Recently, an immunogenic *Anopheles gambiae* salivary protein (gSG6) was identified and proposed as the basis of an immuno-assay determining exposure to Afrotropical malaria vectors. In the present study, IgG responses to gSG6 and 6 malaria antigens (CSP, AMA-1, MSP-1, MSP-3, GLURP R1, and GLURP R2) were compared to anopheles exposure and malaria incidence in a cohort of children from Korogwe district, Tanzania; an area of moderate and heterogeneous malaria transmission. Anti-gSG6 responses above the threshold for sero-positivity were detected in 15% (96/636) of the children, and were positively associated with geographical variations in *Anopheles* exposure (OR 1.25, CI 1.01-1.54, p=0.04). Additionally, IgG responses to gSG6 in individual children showed a strong positive association with household level mosquito exposure. IgG levels for all antigens except AMA-1 were associated with the frequency of malaria episodes following sampling. gSG6 seropositivity was a strong positive indicator of subsequent malaria incidence (test for trend p=0.004), comparable to malaria antigens MSP-1 and GLURP R2. Our results show that the gSG6 assay is sensitive to micro-epidemiological variations in exposure to *Anopheles* mosquitoes, and provide a correlate of malaria risk that is unrelated to immune protection. While the technique requires further evaluation in a range of malaria endemic settings, our findings suggest that the gSG6 assay may have a role in the evaluation and planning of targeted and preventative anti-malaria interventions.

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TAXIS BOXES DETECT LONG-DISTANCE DIRECTIONAL MOVEMENT OF MOSQUITOES TO OLFACTORY CUES

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Malaria control methods targeting indoor-biting mosquitoes are increasingly compromised by insecticide resistance and have limited impact on vectors that feed and rest outdoors. Exploiting mosquito olfactory behaviour to reduce blood-feeding outdoors might be an evolutionarily sustainable approach to complement existing control strategies. Methodologies that can objectively quantify long-range responses to odour under realistic field conditions and allow high-throughput screening of many compounds are required for development of effective odour-based control strategies. The olfactory responses of A. arabiensis females (N = 1920/treatment) in an outdoor field setting to four treatments at four distances up to 100 metres were measured using three-chambered taxis boxes that allow mosquito responses to natural or experimentally-introduced odour cues from the surrounding environment to be quantified under field conditions. Taxis box assays reliably detected directional movement of mosquitoes with more being attracted to natural and blended synthetic human odours than negative controls. However, CO2 alone elicited no response in A. arabiensis. Attraction to stimuli decreased with increasing distance away from the point of stimulation. The range of attraction of mosquitoes to synthetic and human odour extended to 77 and 103 metres respectively. We have developed a reproducible and simple system to allow for the comparison of compounds that are active over medium- to long-ranges in a full field environments. The long natural range of attraction of anopheline mosquitoes to potential blood sources has substantial implications for the design of malaria control strategies, and adds to the understanding of long-distance olfactory behaviour in mosquitoes. While such investigations of malaria vector ecology have enormous potential in their own right, this experimental strategy could also be applied to other motile arthropods of medical, veterinary and agricultural significance.

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A PROSPECTIVE STUDY ON POTENTIAL CAUSES OF DIARRHEA IN BANGLADESHI CHILDREN IN THE FIRST YEAR OF LIFE USING A PCR-LUMINEX BASED DETECTION OF 28 MOST COMMON ENTEROPATHOGENS

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We developed a PCR-Luminex based assay to detect 28 most common organisms associated with diarrhea. The enteropathogens included were *Cryptosporidium spp.*, *Entameoba histolytica*, *Giardia*, Microsporidia, *Cystoisospora, Cyclospora, Ascaris*, Hookworms, *Strongyloides, Trichuris, EAEC*, *EHEC*, *EIEC*, EPEC, ETEC, *Salmonella* (pan), *Campylobacter* (*C. coli* and *C. jejuni*), *Shigella* (pan), *Yersinia* (pan), and *Vibrio* (*V. cholera* and *V. parahaemolyticus*), Astrovirus, Norovirus G1 and G2, Rotavirus, Sapovirus, Adenovirus, and an internal control. This panel was used in a prospective study to determine etiology of diarrhea in Bangladeshi children in the first year of life. Specific primers and probes were designed for the organisms using targets which are conserved. DNA or RNA purified from stool was amplified using biotinylated primers, followed by hybridization to amine-modified probes covalently linked to carboxylated spectrally-distinct microspheres, followed by addition of streptavidin PE to detect specifically-bound amplicon. Luminex results are reported as corrected mean fluorescence intensity (cMFI) normalized to background, where cMFI > 2.5 was utilized as a "present" call with the exception of *Cryptosporidium*, *Strongyloides*, all *E. coli*, and all viruses where cMFI values were 7.3, 9.0, 10.0, and 2.0, respectively. Performance of the assays yielded 95% to 100% sensitivity and specificity versus the assays performed via real-time PCR or other conventional PCR. We then applied the tests in a prospective study of 147 children from Mirpur, Bangladesh followed monthly for the first year of life. 83% of the children had at least 1 episode of diarrhea in the first year of life and 33% had 4 or more. The number of pathogens that were detected increased as the number of diarrheal episodes increased. Rotavirus, *Giardia*, *Campylobactor*, and *Trichuris* were the leading pathogens detected during the first diarrheal episodes, while Adenovirus, *Campylobactor*, *Shigella*, *ETEC*, *EPEC*, and *Giardia* emerged as common in the later episodes. This Luminex based assay for the major enteropathogens offers sensitive and specific detection similar to real time PCR. When applied to field studies in endemic areas, a singular etiology of diarrhea is difficult to determine due to the frequency of mixed infections, and multiple pathogens may be the norm. Pathogens appear to accumulate in children that develop recurrent diarrhea.

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CHARACTERISTICS AND RISK FACTORS OF MODERATE-TO-SEVERE DIARRHEA OF PROLONGED OR PERSISTENT DURATION AMONG CHILDREN LESS THAN FIVE YEARS OLD IN RURAL WESTERN KENYA ENROLLED IN THE GLOBAL ENTERICS MULTICENTER STUDY (GEMS), 2008-2011

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We examined characteristics and risk factors for prolonged or persistent diarrhea among Kenyan children <5 years old participating in GEMS. Children presenting at a clinic with acute moderate-to-severe diarrhea were enrolled. Data on diarrhea duration were reported by the child's caretaker at enrollment for the previous 7 days and were recorded for 14 days after enrollment. Children were measured for weight and height at enrollment and at a 60-day follow-up, and stool specimens were tested at enrollment. We defined an episode of acute diarrhea (AD) as ≤6 days, prolonged acute diarrhea (ProAD) as 7-13 days, and persistent diarrhea (PD) as 14-20 days; the end of an episode was defined by 2 consecutive diarrhea-free days. We conducted logistic and ordinal logistic regression analysis; it was limited to cases with complete data on diarrhea duration and a single episode of diarrhea. From January 31, 2008 to February 6, 2011, 841 children met these criteria. Of these, 494 (59%) had AD, 285 (34%) had ProAD, and 62 (7%) had PD. Infants (OR: 2.0, CI: 1.4-2.8) and toddlers (OR: 1.9, CI: 1.3-2.8) were more likely to have diarrhea of longer duration as compared to 3-5 year olds (*Referent*). Longer durations of diarrhea were more likely to have occurred among children who were offered less than usual to drink while ill (OR: 1.3, CI: 1.0-1.8) and those who were moderately wasted at follow-up (OR: 1.9, CI: 1.1-3.1). Children who were stunted at enrollment (OR: 2.2, CI: 1.3-3.9) were more likely to have PD. Among children with a single pathogen identified (n=331) in their stool specimen, children with *Cryptosporidium* were more likely to have PD (OR: 4.5, CI: 1.3-13.6). Conversely, *Giardia* infection (OR: 0.4, CI: 0.2-0.8) was associated with diarrhea of shorter duration. Diarrhea duration appears to be multifactorial, influenced by a child's age, nutritional status, feeding practices while ill, and the infectious agent. Improved nutrition and appropriate hydration during illness may reduce the consequences and length of a diarrheal episode.

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SEQUELAE OF MODERATE-TO-SEVERE DIARRHEA AMONG YOUNG CHILDREN IN WESTERN KENYA, 2008-2011

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Diarrheal disease remains a major cause of morbidity and mortality among children in developing countries. Diarrheal episodes may predispose children to other illnesses like respiratory infections, but the risk and nature of these complications are poorly quantified. We assessed the risk of various sequelae following an episode of moderate-to-severe diarrhea (MSD) in Kenyan children using data from the Global Enterics Multi-Center Study (GEMS), a 3-year prospective case-control study of children <5 years old presenting at a health facility with MSD (≥3 loose stools in the last 24 hours, within 7 days of illness onset, with ≥1 of the following: sunken eyes, skin tenting, dysentery, IV rehydration, or hospitalization). Control children were matched on age, sex, and village and were diarrhea-free during the 7 days before enrollment. We assessed children at home 60 days after enrollment to obtain information on intercurrent illnesses and complications. We calculated matched odds ratios (mOR) adjusted for age using conditional logistic regression. Between January 2008 and January 2011, 1,473 case and 1,883 control children were enrolled. Within 60 days of presentation with diarrhea, case-patients were significantly more likely than controls to report experiencing an additional diarrheal episode (mOR 3.4, 95% CI 2.9 - 4.1, p<0.0001), dysentery (mOR 2.6, 95% CI 1.5 - 4.9, p=0.0006), or a seizure (mOR 1.8, 95% CI 1.1 - 3.1, p=0.03), and to report cough with difficulty breathing or a clinical diagnosis of pneumonia (mOR 1.3, CI 1.1 - 1.5, p=0.007) or to report being diagnosed with malaria (mOR 1.2, 95% CI 1.0 - 1.4, p=0.01). Additionally, case-patients were more likely to have a weight-for-height z-score <-2 (mOR 2.0, 95% CI 1.6 - 2.6, p<0.0001) or height-for-age z-score <-2 (mOR 1.2, 95% CI 1.1 - 1.4, p=0.003) at follow-up. Increasing diarrhea prevention efforts and careful follow-up of children with MSD may have broad and substantial impact on child morbidity and mortality in developing countries.

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CRYPTOSPORIDIUM INFECTION IN CHILDREN LESS THAN FIVE YEARS OLD WITH MODERATE-TO-SEVERE DIARRHEA IN RURAL WESTERN KENYA, 2008-2011

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Recent data from the Global Enterics Multicenter Study (GEMS), a 3-year case-control study of children <5 years with moderate-to-severe diarrhea (MSD) in seven countries, suggest that *Cryptosporidium* is a major contributor to infant diarrheal illness in Africa and Asia. GEMS cases were children <5 years who presented at a clinic with MSD (≥3 loose stools in the last 24 hours, within 7 days of illness onset, with ≥1 of the following: sunken eyes, skin tenting, dysentery, IV rehydration, or hospitalization). Controls were age-, gender-, and village-matched, and diarrhea-free during the 7 days before enrollment. Stool samples were tested at enrollment; data on water, sanitation and hygiene were collected, and each child's health status was recorded at a 60-day follow-up interview. Presence of diarrhea was recorded by caretakers for 14 days following enrollment. From January 31, 2008 to January 29, 2011 in the rural GEMS Kenya site, *Cryptosporidium* was identified in 160 (10.8%) case children and 88 (4.7%) controls. Prevalence of *Cryptosporidium* was 10.6% among cases <6 months old, 16.2% among cases 6-11 months old, and 8.1% among cases 12-59 months old. Among cases with a single pathogen identified, those with *Cryptosporidium* had a significantly longer median duration of diarrhea than those with another diarrheal pathogen (7.0 vs. 5.0 days, p<0.01), and tended to need hospitalization more often than those with another diarrheal pathogen (17.8% vs. 12.3%, p>0.05). At follow-up, death within 60 days had occurred in 7 (4.6%) of 152 cases with *Cryptosporidium* compared with 45 (3.6%) of 1,267 cases without *Cryptosporidium* (p >0.05). Cases from homes that used rainwater as their main source of drinking water had lower odds of *Cryptosporidium*

infection than cases who used other sources (Odds Ratio = 0.58; 95% CI: 0.40-0.85). Cryptosporidiosis causes a substantial burden of diarrheal illness in young children in Kenya. An intervention aimed at reducing the burden of this pathogen is warranted, such as ceramic filtration of household drinking water.

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SHORT- AND LONG-TERM EFFECTS OF DIARRHEA ON WEIGHT AND LENGTH IN EARLY CHILDHOOD

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The short-term effect of diarrhea on weight faltering is well-accepted, but the long-term effects of diarrhea on both weight and length are less clear. Using data from seven cohort studies, we studied the lagged effect of diarrhea on weight and length in the first two years of life. Our analysis included 1,202 children with 741,846 child-days of diarrhea surveillance and 21,915 length and weight measurements. Using this large, multi-site dataset, we have calculated the effect of experiencing at least one day of diarrhea each month during different time periods on length at 18 months of age. The cumulative effect of monthly diarrhea on length at 18 months of age was -0.58 cm (95% CI -1.11, -0.05). Diarrhea in each of the first 12 months of life was associated with 0.64 cm less attained length (95% CI -1.2, -0.08) when compared with children with no diarrhea during the entire period. There was no statistically significant effect of diarrhea in a specific month of age on length at 18 months of age. Diarrhea in the 30 days prior to the anthropometric measurement was consistently associated with lower weight at most ages, although the association in the early months (1,2, 3, and 5) was not significant. There was little indication of a lagged short-term effect of diarrhea on length. In this large, multi-site dataset that included frequent anthropometry measurements and complete diarrhea histories, diarrhea had small, but measurable, long-term effects on linear growth when burdens were high. These findings support a focus on prevention of diarrhea as part of an overall public health strategy to improve child health and nutrition; however, other factors, such as dietary sufficiency, may be more important to overall linear growth.

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LESSONS LEARNED FROM A SECULAR TREND OF DIARRHEAL DISEASES, RISK FACTORS AND NUTRITIONAL STATUS IN COHORT STUDIES OF CHILDREN IN NORTHEAST OF BRAZIL

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Diarrheal diseases and its associated malnutrition are common causes of childhood morbidity and mortality in developing countries. This study was undertaken to describe changes over time in the incidence of diarrheal diseases, risk factors and prevalence of malnutrition among children in an urban Brazilian community from 1989 to 2012. A secondary aim was to examine the number of episodes of acute diarrhea (AD, duration 13 days) episodes of diarrhea and its impact on children nutritional status. We conducted approximately 12-year (Aug-89 to Mar-00) and 2-year (Jun-10 to Mar-12) cohort studies of 415 and 233 children, respectively, from a Brazilian urban community who were followed from birth. Data were collected on demography, twice weekly surveillance for diarrhea, risk factors and anthropometry. We observed a decline on both episodes and days/child-year over 23 years of follow-up and were correlated with improvements in z-scores nutritional status. The number of AD episodes (≥3 AD episodes) were associated with an increased risk for ProD as well as PD episodes with nutritional consequences. The major protective factors associated with decline diarrheal diseases were increased years of mother education, increased days of exclusive or total breastfeeding practice and better sanitation, especial increased proportion of flush to piped sewer system. Other beneficial factors were greater immunization average coverage for measles and introduction of rotavirus vaccination, better living condition and household income. These results demonstrate marked changes over time in the diarrheal diseases and nutritional status. Number of AD episodes (≥3 AD episodes) also pose increased risk for significant morbidity and identifies children at risk for ProD and PD episodes and the consequences for the vicious cycle of diarrhea and malnutrition, as well as the chronic process of enteropathy. Further studies are needed to understand the etiology, risk factors and pathophysiology of AD episodes burden.

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DEMOGRAPHICS, RESISTANCE PATTERNS AND CLINICAL CHARACTERISTICS OF PATIENTS WITH DIARRHEA AND NON-TYPHOIDAL *SALMONELLA* DETECTED IN STOOL PRESENTING TO A DIARRHEAL HOSPITAL IN DHAKA, BANGLADESH

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Non-typhoidal *Salmonella* (NTS) is a cause of gastroenteritis worldwide, with high rates of invasive disease in sub-Saharan Africa. Data on NTS infection in South Asia are sparse. We used data gathered prospectively from 1996 to 2011 as part of a surveillance system at the Dhaka Hospital of icddr,b, in which every 50th patient undergoes microbiologic evaluation of stool and is queried regarding demographics and clinical manifestations. We identified 468 diarrheal patients in whom NTS were detected in stool, representing 1.3% of all patients surveyed. Over the study period, the frequency of stools positive for NTS decreased (1996-2000, 1.6%; compared to 2007-11, 0.9%; P<0.0001). Highest rates of detection of NTS occurred in the monsoon season (1.5-2.1% of patients, July-Sept), with the lowest rates in the cooler dry season (0.6-0.9%, Jan-Apr). About 47% (219) of total cases of NTS were in children <5 years of age, though when expressed as % of patients, those ≥55 years had the highest rate of NTS isolated (2.2%). Over the study period, rates of resistance to ampicillin, chloramphenicol, TMP/SMX, and ceftriaxone decreased, while rates of decreased susceptibility to ciprofloxacin increased. Among 345 patients with NTS as the sole pathogen identified in stool, 93% reported watery stool, 46% >10 stools in the 24 hours prior to presentation, 78% vomiting, and 48% abdominal pain. Of those <5 years of age (n=162), 41% were stunted and 48% underweight. 16% of patients presented with severe dehydration, 54% with lethargy or drowsiness, and 10% with a recorded presentation temperature of >37.8°C. 23% required IV rehydration and <1% died (n=2). Routine evaluation for bacteremia is not part of the surveillance system, but NTS is not a common cause of bacteremia at the icddr,b (0.6% of all positive blood cultures in 2009-2011). In conclusion, NTS is isolated as the sole pathogen in stool cultures of cases of gastroenteritis requiring hospital care in Dhaka, Bangladesh, with a high burden in malnourished young children and the elderly, but is not a common cause of bacteremia.

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GENES OF THE ANGIOGENESIS PATHWAY ARE ASSOCIATED WITH DEVELOPMENT OF HYDROCELE IN LYMPHATIC FILARIASIS - A CANDIDATE-GENE ANALYSIS

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Of the ~1 billion people at risk in endemic countries, >120 million people are estimated to be infected with *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori*, the causative agents of lymphatic filariasis (LF). Studies have shown that susceptibility to infection, parasite load and lymphatic pathology cluster in families but only a few studies have looked for genes associated with LF and its different clinical presentations, mainly lymphedema and hydrocele. To elucidate the genetic basis of- and possible genetic markers for hydrocele development, which affects 50% of infected men, we performed a candidate gene analysis with 850 *Wuchereria bancrofti* infected volunteers by genotyping 48 single nucleotide polymorphisms (SNPs) in 32 angiogenesis genes by MassARRAY. We found variants in four genes of the angiogenesis pathway significantly associated with hydrocele patients compared to infected individuals without pathology. A SNP in the coding region of the Endothelin-1 gene was associated with pathology (rs5370, *P*=0.015) and formed a haplotype with a SNP in the promoter region (rs1800541, corrected haplotype *P*=0.020). An intron SNP of Caveolin-1 (rs4730748, *P*=0.007) was associated with hydrocele and formed a haplotype with a second intron SNP (rs926198, corrected haplotype *P*=0.002). A SNP in the collagen type 1 alpha 1 gene (COL1A1) and the matrix metalloproteinase 2 gene (MMP-2) were also associated with hydrocele pathology (COL1A1 *P*=0.028; MMP-2 *P*=0.042). To functionally characterize the SNPs, plasma from the study participants has been measured for systemic levels of the respective proteins to be correlated with genotype and expected phenotypes. Our results underscore the fact that hydrocele pathology due to LF has a complex genetic basis, i.e. it is multi-factorial. Further characterization of these 4 angiogenesis pathway SNPs may result in a predictive screen to help physicians prevent pathology development or lead to therapies to ameliorate existing pathology.

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IS THERE BLINDING ONCHOCERCIASIS IN UGANDA? EVIDENCE FROM PADER DISTRICT IN NORTHERN UGANDA

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Onchocerciasis caused by a filarial nematode *Onchocerca volvulus* is transmitted by female black flies of Genus *Simulium* (Diptera: Simuliidae) which breeds in fast flowing rivers. The most common manifestations of onchocerciasis in Uganda are nodules and onchodermatitis. There is virtually no information on ocular lesions. In 2010, a short study on *Simulium damnosum* transmitted onchocerciasis was conducted in Pader district, Northern Uganda. One of the study's main objectives was to determine whether onchocerciasis in this region is the blinding type, with a view to determining the appropriate treatment strategy. A total of 675 persons from 13 randomly selected parishes were examined for clinical, parasitological and ocular manifestations of onchocerciasis. The prevalence of microfilaria in skin snips was 28%, while that of nodules and onchodermatitis was 30% and 29% respectively. The most common skin lesion was Chronic Papular Onchodermatitis (17.5%). However, the prevalence of microfilariae of *O. volvulus* in the anterior chamber of the eye and reversible ocular lesions was 4% each. The reversible ocular lesions of onchocerciasis observed were punctate keratitis stage B (0.1%), punctate keratitis stage D (0.1%) and punctate keratitis stage E (3.7%). On the other hand, 16.1% of the individuals examined had irreversible ocular lesions attributed mainly to Optic atrophy (6.4%) and sclerosing keratitis (5.2%). Visual impairment was detected in 29.2% of those examined and most were due to cataracts (27%) and Optic Atrophy (26%). There was significant association between irreversible onchocercal lesions and visual loss ($p< 0.0001$) and irreversible lesions and nodules ($p< 0.0001$). This study confirms for the first time the occurrence of blinding strains of *O. volvulus* in northern Uganda. It is recommended that treatment [Mass Drug Adminstration (MDA)] be adminstered semi-annually rather than once per year, the current norm. Vector control measures should be instituted to reduce the burden of onchocerciasis, especially visual impairment leading to blindness.

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MASS DRUG ADMINISTRATION: POTENTIAL FOR REVERSING SUBCLINICAL LYMPHATIC PATHOLOGY IN *WUCHERERIA BANCROFTI* INFECTION

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The occurence and importance of childhood lymphatic pathology in filariasis endemic areas is being increasingly recognized. We examined the impact of treatment with Albendazole and DEC, drugs used in Mass Drug Administration (MDA), on lymphatic pathology in100 *Wuchereria bancrofti* infected symptomatic and asymptomatic children (5-18 years) from filarial endemic villages of Odisha, India. 52 children were asymptomatic while the others had early lymphedema, hydrocele or lymphangitis. After clinical examination and screening for microfilaremia and antigenemia (Og4C3 antigen) they underwent lymphoscintigraphy using Tc99 sulphur colloid to detect lymphatic pathology in lower limbs and ultrasonography for detection of adult worms. Lymphatic pathology (visualization of lymphatic tract, non visualization of inguinal nodes, and low tracer uptake ratio at inguinal level at unit time and presence of collateral lymphatic channels in contra lateral limb) was identified in 63 children of whom 29 were asymptomatic. Adult worms were detected in 9 children. Children were randomised to receive either annual (n=49) or biannual (n=51) dose of DEC (6mg/kg) and Albendazole (400 mg) and followed up at six monthly intervals when investigations were repeated. Follow up of 50, 31 & 20 children at 6, 12 & 18 months respectively has been completed. We observed a clinically significant impact on the lymphatic pathology even as early as 6 months, with improvement in the appearance of lymphatics, loss of co-laterals and flow improvement. Improvement in lymphatic flow was observed in 21/26, 16/18, & 9/10 children at 6, 12 & 18 months respectively; while complete reversals were observed in three children at the 12 month follow up. Our findings while demonstrating that treatment can reverse pathology of both early symptomatic and asymptomatic children reiterate the importance of treating early, when there is the possibility that pathology can be reversed. They also provide a powerful message that could be utilized to strenghten the MDA advocacy efforts of the elimination programme.

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SHORTENING THE TIMEFRAME AND DOSAGE OF ANTI-*WOLBACHIA* THERAPY: DOXYCYCLINE ALONE VERSUS DOXYCYCLINE PLUS RIFAMPICIN IN THEIR EFFICACY AGAINST LYMPHATIC FILARIASIS; A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED TRIAL

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The current standard for the treatment of lymphatic filariasis (LF) using antibiotics to target *Wolbachia* endosymbionts is doxycycline (DOX) 200mg/d for 4 weeks, with high macrofilaricidal activity. To test the efficacy of a reduced dosage of DOX to 100mg or shortening the treatment period using a combination of DOX 200mg and rifampicin (RIM) 10 mg/kg/d, a field trial was conducted as part of the A-WOL program. In a Ghanaian area endemic for LF, men were screened by ultrasound for live adult worms (filarial dance sign, FDS). 301 men with FDS were randomized into seven treatment arms (4 weeks (w) DOX 200mg/d, 5w DOX 100mg/d, 4w DOX 100mg/d, 3w DOX/RIM, 2w DOX/RIM, 10 days DOX/RIM, 5w placebo). 254 men completed the treatment per protocol. After four months the participants received ivermectin plus albendazole. Outcome measures were absence of FDS (macrofilaricidal effect) as well as absence of microfilariae (Mf; long-term sterilizing effect). The 5 weeks DOX 100mg/d group showed 75%, 89% and 88% macrofilaricidal activity (absence of FDS) at 12, 18 and 24 months, respectively, compared to 71%, 76% and 83% in the standard and 37%, 36% and 48% in the placebo groups, i.e. showing superiority to placebo and equivalence to standard therapy at all time points. 86% macrofilaricidal activity was also reached with 4w DOX 100mg/d at 18 months. Compared to placebo, significantly fewer men treated with DOX alone or 3w DOX/RIM were Mf positive at 12 months. In the 4w and 5w DOX 100mg/d arms, this effect persisted until month 18 and 24, respectively. The reduction of the DOX dosage from 200mg/d to 100 mg/d (4 or 5 weeks) resulted in >85% macrofilaricidal activity and a long-term sterilizing effect at 18 months, and therefore should be recommended for treatment of LF. This dosage and regime is now equivalent to that recommended for malaria prophylaxis in travellers and for acne. Depending on the favored outcome, a shortened treatment period with the combination of DOX and RIM may be chosen to achieve a long-term sterilizing effect for 12 months.

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ORAL ADMINISTRATION OF A NEW FORMULATION OF FLUBENDAZOLE SHOWS EFFICACY IN THE FILARIAL INFECTION *LITOMOSOIDES SIGMODONTIS*

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Flubendazole is a macrofilariacidal and has been shown to be active in many species including humans. As a very insoluble chemical it has been a challenge to develop formulations that achieve efficacious plasma levels by oral administration. An orally administered amorphous solid dispersion form of this benzimidazole was seen to have a significant effect on the viability, worm load and pathology on a natural infection of jirds with *Litomosoides sigmodontis*. Initially plasma levels of flubendazole were measured in jirds administered 2, 5, and 20 mg/kg. Peak plasma level was at 2 hours and was nearly cleared by 8 hours. Jirds infected with *L. sigmodontis* were administered 0, 2, 6, 20 mg/kg of flubendazole orally once a day for 5 days and then sacrificed at 8 weeks. Tissue pathology was scored, identifiable worms from the peritoneal and pleural cavities isolated and counted, and MTT assay carried out on the isolated worms. A dose response for tissue pathology, worm burden and MTT assay were observed. This study represents one of the first descriptions of the filariacidal activity of flubendazole when administered I orally. The possibilities this approach to the MDA for filarial control and eradication programs will be will be discussed.

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ANTI-*WOLBACHIA* CONSORTIUM (A•WOL) DRUG DISCOVERY - SCREENING OF FOCUSED ANTI-INFECTIVE LIBRARIES FOR NOVEL COMPOUNDS WITH EFFICACY AGAINST *WOLBACHIA* ENDOSYMBIONTS OF FILARIAL NEMATODES

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Filarial nematodes are an important group of pathogens infecting 150 million people throughout the tropics with over 1.5 billion at risk. Control of filariasis currently relies on mass drug administration (MDA) programmes using drugs which principally target the microfilarial life-cycle stage. Antibiotic targeting of filarial *Wolbachia*, an essential bacterial symbiont, provides a novel treatment with macrofilaricidal activity. In order to turn this therapy into a public health tool suitable for filariasis control programmes, the Anti-*Wolbachia* Consortium (A•WOL) utilises *in vitro* cell and nematode assays, followed by secondary *in vivo* assays, to screen both focused and diversity compound libraries against *Wolbachia*. Here we describe the screening of 5399 compounds, from five focused anti-infective chemical libraries, in a *Wolbachia* cell-based assay. We have identified 263 hits, with 117 compounds showing improved efficacy over doxycycline against *Wolbachia*. Hit compounds also show activity against nematode *Wolbachia in vitro* without exhibiting direct anti-nematode activity. Based on our hit criteria, 104 compounds have progressed down the screening funnel and been screened in a *Litomosoides sigmodontis* mouse model, where encouragingly, a number show equivalent or improved efficacy compared with doxycycline against *Wolbachia in vivo*.

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SUCCESSFULLY MANAGING SERIOUS ADVERSE EVENTS (SAES): LESSONS LEARNED FROM NEPAL'S LYMPHATIC FILARIASIS ELIMINATION PROGRAM

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Sixty of Nepal's 75 districts are considered endemic for lymphatic filariasis (LF), putting an estimated 25 million people at risk for contracting LF. In 2002, the Government of Nepal (GoN) formulated a National Plan of Action (2003-2015) for elimination of LF by 2018. Forty six LF-endemic districts have begun LF control programs through mass drug administration (MDA) of diethylcarbamazine and albendazole. These campaigns, targeting entire communities, are implemented by GoN with support from international donors. GoN started LF MDA as a pilot in one district in 2003 and scaled it up to 5 districts in 2006. Among

the Serious Adverse Events (SAEs) reported during this MDA were 15 deaths. Though an independent investigation team confirmed that none of these deaths were associated with MDA, the deaths caused deep concern in the community. Following this event, GoN focused on coordination and advocacy to establish community trust in MDA, and there was no MDA during 2007. With proper advocacy and coordination, the LF MDA restarted in 2008 and was scaled up to 36 districts in 2011. About 250 SAEs including 8 deaths were reported during the 2011 LF MDA. Widespread negative media coverage created fear about MDA within communities, even though an investigation team did not find an association between MDA and deaths. The team recommended a number of actions for future MDAs to minimize and properly manage SAEs. Based on these recommendations, GoN revised its guidelines and emphasized obtaining political commitment and engaging the media and communities for increased advocacy. Efforts were made to increase coordination among health providers, set up response teams in health centers, establish referrals for possible SAEs, and ensure timely response to SAEs. This approach enabled GoN to gain back community trust in the program and there was no interruption in the 2012 LF MDA. Similar efforts by governments and entities implementing MDA in settings beyond Nepal can ensure that SAEs do not hamper program implementation and successes achieved.

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ORIGIN, DIVERSITY AND MOLECULAR CHARACTERIZATION OF A NOVEL PROTEIN-CODING GENE FAMILY WITH SIMILAR SIGNAL SEQUENCE IN *SCHISTOSOMA JAPONICUM*

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Evolution of novel protein-coding genes is the bedrock of adaptive evolution and acquisition of novel molecular functions. Recently, we identified a novel protein-coding gene family with similar promoter region and signal sequence from S. japonicum using signal sequence trap (SST) that isolates secreted and membrane bound molecules. Here, we adopted an integrated approach utilizing bioinformatics and molecular tools to delineate the underlying mechanisms of this phenomenon; and performed functional characterization of the candidate genes, which we found exclusively expressed in S. japonicum. Our analyses and southern hybridization results suggest disperse gene duplication mechanism as a product of DNA-level recombination, mediated by repetitive elements (REs) as inferred from the observed flanking REs (RTE-SJ and Perere classes) around the duplicated gene loci. To investigate the possibility of selective pressure acting on the evolving genes, we sequenced the coding sequences of the genes from the genomic DNA of some strains of S. japonicum, and as expected, identified a significant balancing selection pressure, especially on the putative functional domains of the candidate genes. In addition, a stepwise pathway towards functional selection by alternative splicing was established. While no ancestral homolog was found in other organisms, 3D structure screens revealed similar folding pattern with SEA and SEA-like modules common among proteins in carbohydrate rich environment. We will present data on the molecular characterization and immunogenicity of these candidates from our functional assays and protective effect trials. The role of REs as major mediators of DNA-level recombination leading to dispersive duplication is once more highlighted with evidence from our analyses. Our findings contribute to the growing evidence of the role of REs in the generation of evolutionary novelties in organisms' genomes. S. japonicum has wide range of mammalian hosts and produces more severe hepatic pathology than S. mansoni. Evolutionary novelties after its divergence could account for these distinctive characteristics.

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NEW VIEWS OF SCHISTOSOME DIVERSITY

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The *Schistosoma*tidae comprises about 100 species of blood-inhabiting digenetic trematodes with major medical and veterinary significance. Although the family is comprised of 4 subfamilies, one of them, Griphobilharzinae, is represented by only one species from a crocodile that, as shown by molecular studies, is a member of a different family, the Spirorchiidae. This is supported by the recent finding of spirorchiids from Nepalese snails that cluster with *Griphobilharzia*. As presently known, schistosomes are exclusively parasites of birds and mammals. For *Schistosoma*, relationships among species have been relatively well-defined, including the realization that *Orientobilharzia* clusters within *Schistosoma*, suggesting name changes for the latter genus are in order. New molecular data for schistosomes from elephants (*Bivitellobilharzia*) confirm this is a monophyletic genus consisting of two species, one of which is also reported for the first time from the Asian rhinoceros. Our studies indicate that the diversity of avian schistosomes, that collectively cause swimmer's itch, is considerable. Even in relatively arid New Mexico, 10 species of avian schistosomes have been recovered. Increasingly, molecular signatures can be provided enabling the species responsible for dermatitis outbreaks to be identified. One new genus of avian schistosomes has recently been erected, and another is in progress. Additional likely new genera await further study as we have found both cercariae and adult worms that do not match any known schistosome sequences. The derived clade of avian schistosomes reveals evidence of extensive host switching, particularly with respect to the snail host. For example, schistosomes transmitted through freshwater snails can group closely with those transmitted in marine snails. Additional studies are needed to gain a more complete overall understanding of schistosome diversity before it is lost, and to better resolve the deeper phylogenetic relationships among schistosome genera.

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COUNTRY MOUSE-CITY MOUSE: WHEN SCHISTOSOMES COME TO TOWN

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Although often considered a rural disease, schistosomiasis is frequently being observed in urban settings due to internal migration and strained sanitation infrastructures. The neighborhood of Sao Bartolomeu in Salvador, Bahia, Brazil is a focus of urban schistosomiasis. It is a largely poor community divided into 6 microareas (MA) straddling the Cobre River. Here we examined 1213 of all 1508 individuals from three geographically separated MAs for helminth infection. *Schistosoma mansoni* prevalence rates of 21.9%, 24.6%, and 27.6% were observed in MAs 1, 3, and 6, respectively. Eggs were collected by selective sieving from whole stools of 308 infected individuals. Each sample represented the *S. mansoni* population within each host (infrapopulation). DNA extracted from the egg samples was genotyped for 15 microsatellite loci, and genetic differentiation analyses were performed based on population allele frequencies. Only MAs 3 & 6 have been analyzed so far. Moderate differentiation (mean pairwise Jost's D values 0.053 and 0.043, respectively) was observed within infrapopulations. Similarly, D = 0.049 between the 2 MAs. This profile of an urban focus contrasted with those of two rural villages in Bahia separated by 8 km along the Jiquiriçá River.

Here the prevalence was greater at 40%. The pairwise infrapopulation Ds were also greater at 0.08 and 0.12 within the villages and 0.134 between villages. When combined into component populations, D between rural populations was 0.046 compared to 0.004 between the component populations at the urban sites. Slightly lower genetic diversity was seen in urban vs. rural populations (mean effective allele numbers = 3.62 and 3.78 across loci). If infection were due to only in-migration from the many different areas of Bahia represented in São Bartolomeu, the differentiation and diversity might be expected to be greater than that of a single rural area. This may suggest that the primary transmission here is occurring locally.

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EFFECTS OF PRAZIQUANTEL ON GENE EXPRESSION OF MALE AND FEMALE *SCHISTOSOMA MANSONI* ADULT WORMS IN CULTURE

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Schistosoma mansoni is one of the agents of schistosomiasis, a chronic and debilitating disease. Until now, there is no effective vaccine available for schistosomiasis and praziquantel (PZQ) is the drug of choice for large-scale treatment; unfortunately, the mechanisms of action of PZQ so far are not fully understood. We used a microarray platform to measure large-scale gene expression of adult worms that were submitted to and survived PZQ treatment. SAM one-class statistical test was applied and genes were considered as significantly differentially expressed at q-value ≤0.05. Our results show gene expression of female adult worms was dependent on their male-pairing status when treated with PZQ. Noteworthy, separated females had almost 3 times more differentially expressed genes (1,434) than paired females (486), which could be an indication of why paired females are more sensitive to PZQ when compared to separated females, and an indication of alternative networks and escape mechanisms of female worms. Our analysis showed 219 genes commonly affected by PZQ in both separated and paired females; 96% of theses genes showed an inverted expression pattern that was dependent on the pairing status of the females. We also observed differences in gene expression related to the gender of the adult parasite, when comparing paired male and female worms exposed to PZQ. We found that 48 genes were commonly affected by PZQ in paired male and female adult worms, when compared to their non-treated respective controls. Ingenuity Pathway Analysis (IPA) and Text Mining software were used for functional analyses, identifying gene networks that were significantly enriched with differentially expressed *S. mansoni* genes; among them, some had human homologs that are known targets of other drugs, pointing to possible new parasite targets for a combined treatment regimen for schistosomiasis. These results provide important information to understand the differences related to *S. mansoni* susceptibility to PZQ and therefore differences in the possible mechanisms of action of the drug.

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CHEMICAL AND GENETIC BIOLOGY OF *SCHISTOSOMA MANSONI* PHOSPHODIESTERASE 4 - A POTENTIAL THERAPEUTIC TARGET

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Schistosomiasis is an infectious tropical disease caused by the *Schistosoma* blood fluke. Treatment and control of this disease relies on just one drug and there is a growing concern over the possible emergence of drug resistance. To identify new drug targets we employed a qualitative, phenotypic screening platform evaluating *S. mansoni* parasites against a collection of phosphodiesterase (PDE) inhibitors provided by Anacor Pharmaceuticals of Palo Alto CA. We found that a series of cyclic nucleotide phosphodiesterase 4 (PDE4) inhibitors induce parasite hypermotility and eventually death. PDE4 catalyzes the hydrolysis of the intracellular second messenger, cyclic AMP, regulating its concentration in cells. PDE4 inhibitors are under pre-clinical and clinical evaluation to treat a range of disorders, including other tropical diseases. In the free living nematode, *Caenorhabditis elegans*, the same PDE4 inhibitors active against *S. mansoni* also cause hypermotility. Furthermore, a hypermotile phenotype is seen in *C. elegans* mutants deficient in the orthologous pde4 gene (R153.1). The *C. elegans* gene is localized to synapses ¬-a location consistent with a function in neural cAMP signaling pathways that regulate locomotion. These findings set the stage for (i) inquiring whether the hypermotility of C. elegans induced by the Anacor benzoxaborole compounds is indeed caused by inhibition of PDE4 and (ii) whether the human and *S. mansoni* orthologs of C. elegans pde4 can functionally substitute for R153.1 mutants. Accordingly, by "humanizing" and "schistosomizing" the *C. elegans* pde4, we can take advantage of the amenability of *C. elegans* for genetic analyses and drug screens. We will use *C. elegans* as a surrogate worm to select for compounds that inhibit *S. mansoni* PDE4 but not the human counterpart. Data arising from the chemical screens and genetic experimental approaches will be presented.

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INTERACTION WITH THE HEMOGLOBIN DEGRADATION PATHWAY OF *SCHISTOSOMA MANSONI* AS A RATIONALE FOR ANTISCHISTOSOMAL DRUG DISCOVERY

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The common bloodfeeding characteristic of schistosomes and *Plasmodia* has led to studies with antimalarial drugs against schistosomes in recent years. Amongst different chemical structures, two qualified as leads for antischistosomal drug discovery: mefloquine, a 4-quinolinemethanol, and the artemisinins with their distinct peroxidic scaffold. We will present our work with a group of peroxides, the 3-alkoxy-1,2-dioxolanes as well as mefloquine related compounds belonging to 4-pyridinemethanols, 9-phenanthrenmethanols, and related 4-quinolinemethanols. Additionally we will describe the roles of iron and the peroxidic core in drug activity. All candidates were tested against juvenile and adult stages of *Schistosoma mansoni in vitro*. Successful candidates were followed up *in vivo* on *S. mansoni* infections in mice. Three dioxolanes showed promising *in vitro* activity on both stages (IC_{50}'s ≤ 20.1 μM). However, only moderate, non-significant activity was observed *in vivo*. Two dioxolanes showed high *in vitro* activity against E. caproni, a non-blood-feeding intestinal fluke, and additional iron sources did not alter activity on schistosomes, supporting an iron-independent mode of activation. Non-peroxidic analogues lacked activity against both parasites, underlining the necessity of a peroxide

functional group. Amongst nine selected mefloquine-related compounds, two 4-quinolinemethanols (WR7573, WR7930) showed significantly lower IC_{50}s (< 3.5 µM) than mefloquine (11.4 µM) against schistosomes *in vitro*. Mefloquine and WR7930 showed significantly decreased IC_{50}s when incubated in the presence of hemoglobin. High efficacy was observed for enpiroline and WR7930 against adult *S. mansoni* in mice. Enpiroline, WR7930 and mefloquine also showed high *in vivo* activities against *S. haematobium*. In conclusion, among the different arylmethanols tested the 4-quinolinemethanols reveal the greatest potential as starting point for antischistosomal discovery. Regarding the dioxolanes low *in vivo* activities would need to be overcome to identify an antischistosomal lead candidate.

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PRAZIQUANTEL RESISTANCE IS EXPERIMENTALLY INDUCED IN *SCHISTOSOMA JAPONICUM*

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Praziquantel is currently the only drug of choice for treatment of human schistosomiases. It is worrying about that following subcurative treatment, drug resistance may emerge. The purpose of the present study was to investigate the possibility of the emergence of praziquantel resistance in *Schistosoma japonicum* in Mainland China under drug pressure. Following 8-passage subcurative selection, treatment with praziquantel at single oral doses of 300 and 600 mg/kg achieved worm burden reductions of 32.6% and 68.1%, 45.7% and 61.9%,respectively in Jiangsu and Hunan inducing isolates of *S. japonicum*, whereas being 71.5% and 97.4%, 70.8% and 97.5% in the corresponding field isolates without drug selection. It is concluded that *S. japonicum* is able to develop resistance to praziquantel under subcurative drug selection.

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EXTENSIVE DIVERSITY OF *TRYPANOSOMA CRUZI* DISCRETE TYPING UNITS CIRCULATING IN *TRIATOMA DIMIDIATA* FROM CENTRAL VERACRUZ, MEXICO

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Chagas disease (or American trypanosomiasis) is a parasitic disease of major public health importance, caused by *Trypanosoma cruzi*, which presents extensive genetic diversity. The parasite has been classified into six lineages or discrete typing units (TcI to TcVI) and we performed here the molecular characterization of the strains present in Triatoma dimidiata, the main vector in central Veracruz, Mexico. Unexpectedly, TcI only represented 9/33 strains identified (27%), and we reported for the first time the presence of TcII, TcIII, TcIV and TcV strains in Mexico, at a relatively high frequency (13-27% each). Our observations indicate a much greater diversity of *T. cruzi* DTUs than previously estimated in at least part of Mexico. These results have important implications for the understanding of the phylogeography of *T. cruzi* DTUs and the epidemiology of Chagas disease in North America.

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LATENT INFECTION WITH *LEISHMANIA DONOVANI* IN HIGHLY ENDEMIC VILLAGES IN BIHAR, INDIA

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In Visceral Leishmaniasis (VL) asymptomatically infected persons usually outnumber clinically apparent cases by a ratio of 4-10 to 1. Though little is known about their infectiousness, these latent carriers present a potential challenge for the VL elimination effort. The fact that current diagnostic tests have been validated to detect clinical VL cases but not to detect asymptomatic infections further complicates matters. We report population-based data from a cohort study in a highly endemic area in Bihar, India, describing patterns of markers of infection in relation to age and to other known risk factors for clinical VL. We selected eleven highly endemic villages that were part of a larger study in which annual household surveys are being conducted and information on VL and risk factors for VL has been collected. Two rounds of sero surveys were conducted in which blood samples on filter paper were collected for all those aged 2 years and above who were present at the time of both survey rounds and provided informed consent. Samples were tested by DAT and rK39 ELISA. We enrolled 11,399 persons, 8% of whom were reactive to DAT and 7% to rK39 during the first survey round. Of the 9,919 initially sero negatives, 363 (3.7%) had converted to sero positive one year later. Fifty three percent of initially rK39 positives and 25% of initially DAT positives had reconverted to negative at the time of the second survey round. Whereas clinical VL occurs mainly among young adults, seropositivity and sero conversion were more common with advancing age and were not associated with known risk factors for clinical VL. The age pattern observed in seropositivity and seroconversion could be explained by a boosting effect upon repeated exposure to the parasite. More work is needed to elucidate the significance of serological markers of infection and the role of latent carriership of *L. donovani* in VL transmission.

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A WHOLE BLOOD IFN-Γ/IL-10 RELEASE ASSAY AS A MARKER OF ASYMPTOMATIC INFECTION AND DISEASE STATUS IN HUMAN VISCERAL LEISHMANIASIS

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In endemic area, a large number of exposed individual are able to mount a protective cellular immune response against *Leishmania* and either eliminate infection or remain asymptomatic carriers. Leishmanin skin test (LST) or serological methods have been used to document incident infection, but their value as marker has not been firmly established so far. However, a valid marker of infection is crucial for intervention studies on vaccine or vector control and for monitoring of ongoing transmission in endemic area. We investigated the soluble leishmania antigen (SLA) based whole blood assay on a larger cohort of subjects, to detect IFN-γ, TNF-α and IL-10 released cytokines and aimed to correlate with the marker of asymptomatic infection and disease status of infected individuals. We employed the whole blood assay to evaluate IFN-γ, TNF-α and IL-10 production in 35 patients with active visceral leishmaniasis (VL), 54 patients with cured VL, 27 patients with other diseases and 52

Non-Endemic Healthy Controls (NEHC). We also tested the assay on 147 Endemic Healthy Controls (EHC), all close contacts of VL cases having a high probability of *L. donovani* infection, and correlated their cellular response with their serological antibody titers against *L. donovani* and Phlebotomus argentipes saliva. The whole blood IFN-γ release assay had a sensitivity of 85.2% (95% CI 73.4 - 92.3) in cured VL patients to detect the cellular immune response and a specificity of 100% (95% CI 93.1 - 100.0) in NEHC. The assay detected IFN-γ release in 24% of the EHCs, and these individuals also had elevated titers of anti-saliva antibodies, consistent with their having had a higher risk of exposure to an infected sand fly bite. Only the active VL patients produced IL-10, which can therefore be assayed in conjunction with IFN-γ to distinguish active cases from clinically exposed, immune individuals.The findings strongly reinforce the utility of the SLA based whole blood assay for the detection of IFN-γ production by *L. donovani* infected, asymptomatic individuals, and for detection of IL-10 secretion as the signature cytokine distinguishing active VL from cured or asymptomatic cases.

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CHAGAS DISEASE AMONG LATIN AMERICA IMMIGRANTS EVALUATED IN A TROPICAL MEDICINE CLINIC IN BRONX, NEW YORK

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Due to increase awareness, immigration and screening of the blood supply Chagas disease is an important "emerging" infection and public health issue in the United States (US). This study describes a 7 year (2005-2012) experience with Chagas disease in a tropical medicine clinic in NYC. Patients were referred from the Blood Center or from a primary care physician. Antibodies were detected with ELISA and were confirmed with IFA performed at CDC. Baseline blood counts, electrocardiogram (EKG) and echocardiogram (echo) were performed. Twenty-one patients (12 male [57%]), mean age of 45±17 years were evaluated. Patients were from Mexico (6), El Salvador (6), Ecuador (3), Argentina (1), Honduras (1), Bolivia (1) and 3 were born in the US to immigrant mothers. Mean time from immigration was 19±9 years. History of living in a mud house was recalled by 7(44%) patients. Twelve patients (57%) were diagnosed as a result of blood transfusion screening. Abnormal EKG findings were observed in 7 patients, including: sinus bradycardia, first degree AV block, right bundle branch block. Six patients had abnormal echo findings: left ventricle apical aneurysm, wall motion abnormalities, diastolic dysfunction and chambers dilatation. Four (20%) patients, with a mean age of 67±9 years, had advanced cardiac disease; one patient required a transplant. Ten patients were asymptomatic (mean age 4±14 years); 4/6 asymptomatic patients had normal EKG and echo. Six patients (mean age 32±8 years) were treated, 4 with nifurtimox and 2 with benznidazole. All patients developed side effects; cutaneous toxicity was associated with benznidazole, leading to discontinuation of drug; and headaches, insomnia, myalgias and gastrointestinal complaints with nifurtimox. Our experience underscores the importance of screening immigrants who are both asymptomatic or have cardiac abnormalities. US-born children who have never visited endemic areas are also at risk of mother to child transmission and should be screened. Physicians in developed countries should become familiar with the diagnosis of this disease.

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DRUG DISCOVERY ALGORITHM FOR CUTANEOUS LEISHMANIASIS

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The development of new drugs for cutaneous leishmaniasis has been hampered by lack of disease awareness, funding, and drug discovery models that predict clinical outcome. Cutaneous leishmaniasis is clinically widespread but lacks treatment options that are effective and well tolerated. Because all present drugs have been grandfathered into clinical use, there are no examples of a pre-clinical product evaluation scheme that lead to new candidates for formal development. We have developed a testing strategy that features a gated, resource sparing model that progresses from high-throughput *in vitro* promastigote and axenic amastigote assays to more clinically relevant, comparable mouse *Leishmania* suppression and *Leishmania* cure models that have undergone internal validation and are reproducible. The results of our automated, high-throughput screening of potential drugs *in vitro* against promastigotes was recently published and the complimentary screening against axenic amastigotes is detailed elsewhere. Our process for advancing compounds from hit to lead which includes compound throughput and additional compound activity metrics will be discussed.

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USING METABOLOMIC AND PROTEOMIC ANALYSES TO STUDY DRUG RESISTANCE IN *LEISHMANIA INFANTUM*

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Drug resistance in leishmania is extremely prevalent and a huge obstacle to successful treatment of the disease. The mechanisms of resistance are complex and whole genome studies have revealed a huge amount of inter- and intra-species variation. We demonstrate that strains resistant to leishmanicidal drugs exhibit cross tolerance, shown by lack of ROS induction, a lack of membrane potential loss and a lack of DNA laddering, to other leishmanicidal drugs, while retaining susceptibility. Hydrogen sulphide production was analysed and found to be reduced in resistant strains compared to sensitive, which in bacteria was shown to have effects on glutathione reductase and super oxide dismutase expression. Metabolomics is a relatively new tool in the biologist's toolbox, aiming to analyse the small chemical compounds that determine the front end of cell metabolism. By subjecting metabolites from drug sensitive and drug tolerant strains to mass spectrometry and NMR analysis, we highlight changes to the way the parasite's metabolome responds to drugs. These alterations to the response pathways may underlie the selection of cross-tolerant and further multi-drug resistant strains. As many leishmanicidal drugs are targeted towards the mitochondrion of the parasite, we were also interested in characterising quatitatively the mitochondrial proteome in drug sensitive and resistant strains to determine whether the metabolomic changes seen can be explained by differential expression (using RNAseq data) or modifications to these proteins. The new methods for the purification of mitochondria using differential gradients and Zone Free Flow Electrophoresis will be discussed, in addition to the latest results obtained using these methods. By determining the drug combinations which more easily lead to cross tolerance and resistance, we hope to inform the clinical treatment of leishmaniasis and by determining the metabolic mechanisms by which tolerance and cross-resistance occur, we suggest compounds which may ameliorate the action of the drugs.

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NON-SEROLOGICAL DETECTION OF PARASITE BIOMARKERS IN HUMAN BLOOD FOR THE DIAGNOSIS OF CHAGAS DISEASE

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Trypanosoma cruzi, a blood borne pathogen, is the etiological agent of Chagas disease in humans. Following an infection, patent parasitemia is detectable in the blood of infected individuals and this phase is termed the acute phase. This is followed by a chronic phase that persists for the life of the individual. During this phase, 20-30% of infected individuals will develop clinical symptoms. Diagnostic assays for Chagas disease detect host anti-*T. cruzi* antibodies as a surrogate marker for infection. However, these assays are not reliable during the early period when infected individuals have not yet sero-converted. Additionally, serological tests are unreliable for determining treatment efficacy as anti-*T. cruzi* antibodies persist for a long time and their levels do not correlate with parasite clearance. To overcome these drawbacks, we envisaged the detection of antigens secreted by parasites, collectively termed as *T. cruzi* Excreted Secreted Antigens (TESA), as a diagnostic for Chagas disease. We utilized *in-vitro* RNA SELEX methods to develop TESA aptamers (short RNA molecules) with the goal of utilizing them as specific ligands in detection assays. The TESA SELEX was performed using cell culture supernatant from *T. cruzi* trypomastigote infected NIH 3T3 cells. Biotinylated monoclonal aptamers were utilized in a modified enzyme linked assay to detect TESA in sera from Chagasic patients. Patient samples were obtained from endemic areas (Goias and Minas Gerias) of Brazil and were clinically classified as acute and chronic infections. Several aptamers screened showed significant binding to serum from infected individuals compared to non-infected endemic controls. These interactions were specific, as scrambled aptamers did not bind to serum from infected individuals. This is the first demonstration of an aptamer based assay that detects a parasite biomarker for the diagnosis of Chagas disease in humans.

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THE TORORO CHILD COHORT: A LONGITUDINAL STUDY OF MALARIA IN THE SETTING OF INSECTICIDE TREATED BEDNETS AND ARTEMISININ-BASED COMBINATION THERAPY

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Many reports have suggested that malaria incidence is decreasing in Africa in the setting of malaria control interventions, but longitudinal studies are lacking. We enrolled a cohort of 100 HIV negative children 6 weeks to 10 months of age in an area of high malaria transmission intensity in Uganda and followed them through 48 months of age. Children were enrolled during 2007-2008, given LLITN at enrollment, and received all care at a study clinic, including ACTs for episodes of malaria and routine monthly blood smears. Malaria incidence was measured using passive surveillance with incident malaria defined as a fever and positive thick blood smear in the absence of malaria treatment in the prior 14 days. Asymptomatic parasitemia was defined as a positive smear in the absence of fever and not followed by malaria within 7 days. We calculated the relative risk of incident malaria by age, calendar time and season using generalized estimating equations with robust standard errors. 100 children were enrolled (mean age at enrollment 5.52 months) of whom 79 reached 48 months of age; children were followed for a median of 3.46 years. A total of 1633 incident episodes of malaria were observed in this cohort, with a median incidence of 5.32 ppy (IQR 3.16-6.8) despite 98% compliance with LLITNs. There were only 6 cases of complicated malaria, all due to single convulsions. Malaria transmission was year-round, with two annual incidence peaks from Nov-Jan and Apr-Jul, corresponding to rainy seasons. Malaria incidence peaked at 6.5 ppy at 23 months of age before declining to 3.5 ppy at 48 months of age. After adjusting for age and season, the relative risk of malaria increased by 52% from 2008 to 2011 (RR 1.52, 95% CI 1.10-2.09). Asymptomatic parasitemia was uncommon (monthly prevalence <10%), and was rarely detectable prior to 24 months of age. Despite LLITNs and prompt treatment with ACTs, the incidence of malaria is very high in Tororo and appears to be rising. Additional malaria control interventions among young children living in high transmission settings are needed.

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RELATIONSHIP BETWEEN PARASITE BURDEN AND SEVERITY OF MALARIA ILLNESS IN AFRICAN CHILDREN

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Severe *Plasmodium falciparum* malaria is a major cause of pediatric mortality. The contribution of parasite burden to the pathogenesis of severe malaria has been controversial. We documented *P. falciparum* infection and disease in 882 Tanzanians followed from birth up to age 4 years. 102/882 children experienced severe malaria, but only 3 suffered more than 2 episodes. Risk of first severe episode was stable over several infections. Mean parasite levels were higher during severe disease, but overlapped considerably with those of children with mild malaria at the same age. 21/102 children experienced high density infection with no more than mild symptoms before having severe malaria. Severe malaria incidence rapidly decreased after infancy, while incidence of high density infection (>100,000 parasites/µl blood) increased. Mean parasite densities were similar during infections before versus after severe malaria episodes; 55/102 children experienced high density infections afterward with mild symptoms. Bed nets, maternal malaria, malaria season, and sickle trait modified severe malaria risk, and also modified parasite density during infections. Based on these data, resistance to severe malaria is not acquired after 1-2 mild infections. Although parasite burden on average is higher during severe malaria episodes, high parasite burden is often insufficient to cause severe malaria even in children who later prove to be susceptible. The diverging rates of severe disease and high density infection after infancy, and similar parasite burdens before and after severe malaria, indicate that naturally acquired resistance is not related to improved control of parasite density.

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RISK OF READMISSION OR DEATH WITHIN SIX MONTHS AFTER INITIAL DISCHARGE AMONG UGANDAN CHILDREN WITH SEVERE MALARIAL ANEMIA AND CEREBRAL MALARIA

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Severe malarial anemia (SMA) is a leading cause of morbidity and mortality among young children in sub-Saharan Africa. Children with SMA appear to have an elevated risk for re-hospitalization and death during the first 6 months following discharge, but the risk of hospital readmission and death for children with cerebral malaria (CM) has not been assessed. We compared the risk of readmission to hospital or death within 6 months of admission in Ugandan children aged 18 mo - 12 y who were successfully treated for CM or SMA Mulago Hospital, Kampala, Uganda and then followed up for 6 months. Children with sickle cell disease were excluded from this analysis. Eligible children with CM (n=167) or SMA (n=144) were compared to asymptomatic community children (n=160). The primary endpoint, readmission or death, was infrequent in CC (5%), but more frequent in children with SMA (25.7%, P<0.0001) or CM (16.8%, P=0.0007). Readmission or death was higher in children with SMA than children with CM (P=0.07). Assessing individual outcomes, frequency of readmission within 6 months of discharge was higher in children with SMA (21.5 %) or CM (16.2 %) as compared to CC (5%, P<0.0001 for SMA, P=0.001 for CM), but did not differ significantly between children with CM and children with SMA P=0.22). Most hospitalizations were for malaria, though on many hospitalizations no blood smear confirmation was obtained. Frequency of death was also higher in children with SMA (3.4%) as compared to CC (0%, P=0.02) or children with CM (0.6%, P=0.07). Most deaths were reported as due to a febrile illness. Children with SMA and CM have a higher risk of readmission within 6 months after discharge than community children, and children with SMA have a greater risk of death within 6 months of discharge than children with CM or community children. Further study is needed to assess causes of readmission or death in children with severe malaria.

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IDENTIFICATION OF HOTSPOTS OF MALARIA TRANSMISSION IN WESTERN KENYAN HIGHLANDS; PAVING THE ROAD TO TARGETED AND EFFECTIVE INTERVENTION STRATEGIES

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Malaria risk is not uniformly distributed between districts, villages and even households from the same village. Some households are disproportionally exposed to malaria and incidence in these households may be more than 5-fold higher than the village mean. This micro-heterogeneity in the burden of malaria is largely explained by variation in exposure to infected mosquito bites and may complicate malaria control by resulting in large variations in the impact of interventions. Whilst mean levels of malaria transmission may decline in an area, strongholds of intense transmission may persist even during the seasons of low transmission intensity. In the highlands of western Kenya, malaria transmission is highly variable despite universal high coverage rates with insecticide treated nets (ITNs) and indoor residual spraying (IRS). In Rachuonyo South district, community surveys that were conducted in 2010 indicated parasite prevalence averaging 14.8% but ranging from 0% to 51.5% this effect seemed more pronounced in children aged 8-14 years where parasite prevalence was on average 25.8% but ranged from 0 to 71.4%. The true extent of heterogeneity in these areas remains unknown but it is likely to have an impact on effective malaria control. In a recent cross-sectional survey, we attempted to identify micro-epidemiological patterns in malaria transmission in the Rachuonyo South district. We collected blood samples from approximately 3,200 households comprising 17600 individuals, in an area of 100km2. Every household was mapped with a GPS device. Hotspots of malaria transmission were identified by determining spatial patterns in the prevalence of *Plasmodium falciparum* parasites, determined by PCR analyses (23.0% positive, 95% CI 22.1-24.0%) and serological markers of malaria exposure based on the levels of AMA-1 (44.4% positive, 95% CI 43.6-45.2%) and MSP-119 (34.4% positive, 33.7-35.1%), antibodies. Serological markers of malaria exposure have the advantage of providing information on malaria exposure that is less susceptible to seasonal variations in the force of infection. 29.3% of the people (95% CI 28.7-30.0%) lives inside hotspots of more intense malaria transmission as detected by parasite prevalence and density of malaria specific antibodies (p<=0.05). The identified 'hotspots' of malaria were then used for a targeted intervention strategy deployed in March 2012. The impact of this strategy is currently under evaluation.

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THE SPATIAL AND TEMPORAL HETEROGENEITY OF ASYMPTOMATIC *PLASMODIUM FALCIPARUM* PARASITEMIA AMONG KENYAN SCHOOL CHILDREN

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As malaria transmission intensity declines and infection heterogeneity increases, identifying and treating "hotspots" is becoming increasingly important in the fight for elimination of malaria. Community based surveys are logistically complex and costly, but schools could be effective platforms for identifying such "hotspots" if school-based screening demonstrates clusters of infection. This study investigates the spatial and temporal patterns of asymptomatic *Plasmodium falciparum* infection in schoolchildren and the ecological and sociodemographic predictors of such patterns. A cohort of 2400 children from 51 primary schools were tracked at six time points, 2010-2012, as part of a trial investigating the impact of school-based malaria control on the south coast of Kenya. Demographic, socioeconomic and bednet use data were recorded for each child and households of children in 26 schools were mapped. Environmental covariate information was derived from high-resolution satellite data and spatially explicit Bayesian hierarchical modelling was used to examine clustering at school and household levels. Marked geographical heterogeneity of P.*falciparum* infection was observed, with persistently high transmission exhibited in a minority of schools . High rates of reinfection and the clustering of infection across small groups of households over time were observed, and indicate "hotspots" at the local level. The strong infection heterogeneity, persistent over time, points to a role for school-level screening in active detection of clusters in the wider community, and suggest a role for reactive screening and treatment of other members of households of positive cases identified in schools.

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HOUSEHOLD MALARIA PARASITE BIOMASS AND SUBSEQUENT RISK OF SYMPTOMATIC MALARIA IN CHILDREN IN TANZANIA

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Hotspots of malaria transmission are likely to occur at all levels of transmission. One component that supports a hotspot is the presence of semi-immune asymptomatic carriers of malaria parasites that are clustered in households. We hypothesize that this asymptomatic pool of parasites is responsible for increased transmission of malaria. Thus household parasite biomass is a determinant of the risk of symptomatic malaria in children under the age of 5 years. The study was carried out in four villages in Mwanza region, Tanzania, an area with moderate transmission of *P. falciparum*. In a cross sectional survey, carried out in the dry season between August- October 2010. Blood samples to detect parasite biomass at household level were collected from all household members. Passive case detection of symptomatic malaria cases took place at heath facilities located at the study site for one calendar year following the survey. Quantitative Polymerase Chain Reaction (qPCR) was used to determine biomass at the household level. Geospatial analysis was used to define households and clusters with high dry season parasite biomass and logistic regression modeling was used to determine the predictors of subsequent risk of malaria in children under 5 years of age. Children living in households with higher parasite biomass had three times the risk of having a subsequent attack of symptomatic malaria detected by passive surveillance compared to households with low parasite biomass. Clustering of parasite biomass was associated with the incidence of symptomatic malaria. These results support the hotspot theory of malaria and suggest that targeting asymptomatic carriage in households will reduce the incidence of malaria in young children.

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SHRINKING THE MALARIA MAP IN MULEBA DISTRICT, NORTHWESTERN TANZANIA: MONITORING THE IMPACT OF MALARIA PREVENTION INTERVENTIONS SCALE-UP THROUGH HOSPITAL ADMISSIONS

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Muleba district (population of 450,000) in north-western Tanzania, an unstable *Plasmodium falciparum* transmission area, experienced severe malaria outbreaks in 2006/7. In 2007, indoor residual spraying (IRS) was introduced as an outbreak preemptive measure covering about 200,000 people. One IRS round per year was applied in the entire district since 2009. About 80,000 long-lasting insecticidal nets (LLINs) were distributed to under-fives in 2009. In 2011 another 170,000 LLINS were distributed to cover the remaining population. Malaria admissions can be considered a proxy indicator of the occurrence of severe malaria and, hence, intensity of malaria transmission. In 2006 a special surveillance system was established at five health facilities with in-patient services in the district. All patients with a laboratory confirmed diagnosis of malaria were recorded and entered into a database (age, gender, dwelling, disease outcome, date of admission). Between 2006 and 2011 a total of 45,512 cases were recorded and 32,839 (72%) were under-five years of age. The database allowed monitoring and mapping of severe malaria morbidity trends at different intervals and administrative units. Malaria admission rate (number of admission per 1000 population) among under-fives declined from 145 to 20 per 1000 (86% reduction) between 2006 and 2010, with significant variation in decline among district locations. An increased malaria admission rate (70 per 1000) was then observed in 2011 with high variation among the different location from 0/1000 to 99 per 1000. Several factors contributed to this increase, including acute ACT stock-outs in health facilities, decreased susceptibility to the insecticide used for IRS, abnormal precipitation, and low net use. The experience in Muleba demonstrates the importance of surveillance to monitor disease trends after malaria intervention scale-up and to stratify malaria transmission risk to help detect and locate abnormal increases in transmission. Carefully-coordinated surveillance and response are required to address ongoing, low-level transmission hot spots as well as acute outbreaks once control of malaria is achieved.

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SEASONAL MALARIA CHEMOPREVENTION AND COMMUNITY CASE MANAGEMENT FOR MALARIA IN SOUTHERN SENEGAL: A CLUSTER-RANDOMIZED TRIAL

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Although the overall incidence of malaria has recently declined in Senegal as in some other countries, this hides the fact that the burden of malaria remains very high in some parts of the country, such as Saraya district, where 70% of the community lives more than 15km from the nearest health post. Community case management for malaria is being introduced, volunteers (Distributeurs de soins à domicile or DSDOM) are trained to recognize the signs and symptoms of uncomplicated and severe malaria, to use Rapid Diagnostic Tests, and to treat malaria with artemisinin combination therapy. The DSDOM could also deliver Seasonal Malaria Chemoprevention (SMC) to children, SMC is known to be highly effective in preventing malaria illness but the relative advantage of adding SMC in villages which have access to prompt effective treatment from the village health worker has not been evaluated. In this trial, 24 villages were randomized to deliver SMC with community case management, or community case management alone. In SMC villages, the DSDOM gave all children under 10 years old preventive treatment with sulfadoxine-pyrimethmine plus amodiaquine each month from July to November 2011. Previously SMC has been delivered over three months so this study will also provide new evidence about the feasibility, tolerability and acceptability of delivery over a longer period. The DSDOM were trained to make blood films which were collected by the study team so that malaria cases could be confirmed by microscopy. The impact of SMC on drug resistance is being evaluated by analysis of used RDTs and from blood samples taken from a sample of children at the end of the transmission season. The added value of SMC will be discussed, and data on the impact of the intervention on malaria, anaemia and the safety profile of 5 doses of SMC will be presented.

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RANDOMIZED CONTROLLED TRIAL OF LEVAMISOLE HYDROCHLORIDE AS ADJUNCTIVE THERAPY IN SEVERE *FALCIPARUM* MALARIA WITH HIGH PARASITEMIA

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Cytoadherence and sequestration of red blood cells containing the mature stages of *Plasmodium falciparum* are central to the pathogenesis of severe malaria. The oral antihelminth drug levamisole inhibits cytoadherence *in vitro* and reduces sequestration of late stage parasites in uncomplicated *falciparum* malaria treated with quinine. We did an open label randomised controlled trial to assess the benefit of levamisole as adjuvant treatment in adults patients with severe *falciparum* malaria treated with artesunate. A total of 56 adult patients with severe malaria and high parasitaemia admitted to a referral hospital in Bangladesh were randomised to receive a single dose of levamisole hydrochloride (150 mg) or no adjuvant to antimalarial treatment with intravenous artesunate. Main outcome measures were the kinetics of late stage peripheral blood parasitaemia, reversal of lactic acidosis and rectal capillary blood flow by orthogonal polarising spectroscopy. Circulating late stage parasites measured as the area under the parasite clearance curves for late trophozoite and schizont stage parasites was 2150 (0-28,025) parasites/µl*h in patients treated with levamisole versus 5489 (192-25848) parasites/µl*h in controls (p=0.254). The 'sequestration ratios' at 6 and 12 hours for all parasite stages were no different between the groups. The time to normalisation of plasma lactate (<2 mmol/L) was 24 (12-30) hours with levamisole versus 28 (12-36) hours without (p=0.148). There was no benefit of levamisole hydrochloride as adjuvant to intravenous artesunate in the treatment of adults with severe *falciparum* malaria. The potent killing of ring stage parasites by intravenous artesunate, preventing their further maturation and sequestration, might obscure the effects of levamisole in preventing sequestration.

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EVALUATING THE IMPACT OF ENHANCED HEALTH FACILITY-BASED CARE FOR MALARIA AND FEBRILE ILLNESSES IN CHILDREN IN UGANDA: THE ACT PRIME STUDY

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Early diagnosis and prompt effective treatment reduces morbidity and mortality from malaria. However, inadequate health services limit appropriate fever case management in the public sector. We are conducting a cluster randomized trial in Tororo, Uganda to assess whether enhancing lower-level government health centers improves appropriateness of antimalarial treatment and patient satisfaction. Twenty health centers have been randomized; 10 to the intervention, and 10 to standard care. The intervention, which began in May 2011, includes training in health center management, fever case management, and patient-centered services, and provision of rapid diagnostic tests (RDTs) for malaria and artemether-lumefantrine when stocks run low. We are conducting a series of evaluations interviewing caregivers of children under five years as they exit health centers. Information is gathered about the purpose of the visit, diagnostic testing for malaria, diagnosis given, and medications prescribed and received, and satisfaction with the visit to the health center. If the child has fever or history of fever, a RDT is performed. Study outcomes include (1) the proportion of children with suspected malaria who were inappropriately treated for malaria, based on the result of the research RDT, considering those children with a negative RDT who were given artemisinin-based combination therapy (ACT) plus those with a positive RDT who were not given an ACT, (2) the proportion of children with a positive RDT who were inappropriately treated with a non-ACT regimen, and (3) patient satisfaction with their experience. Two rounds of interviews have been carried out, in August 2011 and February 2012. In each survey, caregivers of 200 children were interviewed, including 10 from each facility. Full results from three rounds of interviews will be presented, providing much needed evidence of the effectiveness and sustainability of a complex health facility-based intervention on provider practices and patient management.

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A NEW APPROACH FOR MALARIA CONTROL IN SCHOOLS: RESULTS OF A RANDOMIZED TRIAL OF INTERMITTENT PARASITE CLEARANCE

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Malaria control has traditionally focused on pregnant women and children under five years, in whom the risk of malaria-related mortality is greatest. Yet recent studies have shown that older school-age children could also benefit from malaria control, with potential gains for both health and education. Senegal recently introduced universal coverage of nets, with national roll-out of community-wide distributions starting in 2010. Whilst successful in achieving high levels of coverage amongst school-children in Kedougou, over 30% of children remained infected at the end of the malaria transmission season in December 2010. This calls for additional control measures in this age group. One potential supplementary strategy is intermittent parasite clearance in schools (in which a treatment dose is given irrespective of infection status) with the aim to improve educational performance by reducing malaria-related anaemia and improving cognitive function among schoolchildren. This approach is particularly suited to areas of seasonal transmission where a single annual treatment can be given at the end of the transmission season. To evaluate this approach, a randomized double-blind placebo-controlled trial was conducted amongst children already sleeping under insecticide-treated nets in Kedougou, Senegal. Children enrolled in six primary schools (7-14 years) were individually randomized to either receive IPC with sulphadoxine-pyrimethamine and amodioquine (SP+AQ), or placebo (n=865). Following informed parental consent and baseline parasitological survey, children received a single treatment dose in school at the end of November 2011. IPC treatment was administered over three days by the research team. The impact of the intervention on malaria parasitaemia, anaemia, and tests of sustained attention was evaluated through a transverse survey in February 2012. The advantages and disadvantages of this control approach will be discussed, and data on the impact of the intervention on malaria, anaemia and cognitive function at follow-up will be presented.

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ASSESSING THE EFFECT OF THE RECOMMENDED DIHYDROARTEMISININ-PIPERAQUINE DOSING REGIMEN ON THE RISK OF TREATMENT FAILURE IN PATIENTS DIAGNOSED WITH UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA

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The fixed dose antimalarial dihydroartemisinin-piperaquine (DHA-PQ) is administered according to weight or age banding. To assess whether dosing strategies and total dose of piperaquine were associated with treatment efficacy, individual patient data (N=3573) were collated from 11 clinical efficacy studies of uncomplicated *P. falciparum* conducted between 2003 and 2010 with a follow up of 28 days or longer [Africa:2427, Asia:1146). There were a total of 59 recrudescent and 336 new infections. The spread of PQ dosage was greatest in children <5 years (median=55.6 mg/kg, IQR: 47.5-64.0 mg/kg) compared to patients aged 5-15 (median=55.6 mg/kg, IQR: 51.2-61.9 mg/kg) and >15 years (median=51.1 mg/kg, IQR: 47.4-54.3 mg/kg). Children <5 years were at greatest risk of recrudescence (2.2%, 52/2371) compared to those aged 5-15 (0.9%, 4/457) and >15 (0.4%, 3/745). Patients failing treatment received a lower dose of PQP (median: 48.9mg/kg, IQR: 43.6-52.1) compared to those who were cured (median: 53.3mg/kg, IQR: 48.0-60.0). In the multivariate analysis an overall PQ dose < 48 mg/kg (AHR=1.7 [95% CI: 0.9-3.0], *P=0.0720*) and an age <12 years (AHR: 4.7 [95% CI: 1.3-17.8], *P=0.0220*) were independent risk factor for recrudescence. PQ dose <48 mg/kg was administered in 25.2% (201/2371) of children aged <5 years, 9.4% (43/457) in 5-15 age band and 26.9%(201/745) in those aged >15. In the children <15 years, the failure rate was 1.8 times greater in those who received a mg/kg dose below the 48 mg/kg threshold (3.17%, 20/631) compared to those who received >48 mg/kg (1.67%, 36/2150). The efficacy of DHA-PQ is vulnerable to under dosing particularly in young children. The lower mg/kg dose of PQ in patients who failed treatment and higher failure rates in children suggest that further dosing optimization to ensure adequate efficacy in young children may be warranted.

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IN VIVO EFFICACY OF SULFADOXINE-PYRIMETHAMINE FOR THE TREATMENT OF ASYMPTOMATIC PARASITEMIA IN PREGNANT WOMEN IN MALAWI

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Malaria in pregnancy is associated with severe maternal anemia, placental parasitemia, low birth weight, and increased perinatal mortality, especially among primigravidae. Sulfadoxine-pyrimethamine (SP) is recommended for intermittent preventive treatment in pregnancy (IPTp), but its effectiveness might be compromised by high prevalence of SP resistance. We assessed the *in vivo* efficacy of SP in asymptomatic parasitemic women as proxy for SP effectiveness in this vulnerable population. Pregnant women between 16 and 26 weeks gestation with asymptomatic parasitemia who presented for antenatal care at Machinga District Hospital in Malawi, were given SP and followed for 42 days. Survival analysis was conducted to determine drug efficacy. We included 245 pregnant women of whom 150 reached a valid study endpoint. The survival rate was 64.5% (95% confidence interval (CI) 58.3- 70.3%). Of those who experienced recurrent parasitemia, polymerase chain reaction (PCR) could be done on 78% to differentiate reinfection from recrudescence (13 could not be amplified and 6 were unavailable). Of those with PCR data, 75% had recrudescence. Missing PCR results were assumed to follow a similar distribution with 75% due to recrudescence. The PCR corrected survival was 73.1% (95% CI 67.2- 78.3%). Recrudesence was more common among primi- than among multigravidae, with a recrudescence rate of 33.3% (95% CI 25.1-42.4%) and 21.4% (95% CI 15.0- 29.0%), respectively (log rank test p-value 0.006). SP had low *in vivo* efficacy in asymptomatic parasitemic pregnant women, especially primigravidae. New efficacious antimalarials are needed to prevent malaria in pregnancy.

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ASSOCIATIONS BETWEEN INTERMITTENT PREVENTIVE THERAPY WITH SULFADOXINE-PYRIMETHAMINE AND PLACENTAL MALARIA IN AN AREA OF HIGH ANTIFOLATE RESISTANCE IN TORORO, UGANDA

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Intermittent preventive treatment with sulfadoxine-pyrimethamine during pregnancy (IPTp-SP) is widely recommended in sub-Saharan Africa to reduce the risk of placental malaria (PM) and the adverse birth outcomes associated with this disease. However, there are reports that the efficacy of IPTp-SP is waning, especially in parts of East Africa where antimalarial resistance to antifolate drugs has become widespread. We conducted a cross-sectional study of 565 HIV uninfected women giving birth at Tororo District Hospital, an area of Eastern Uganda with high transmission intensity. The primary objective of the study was to measure the association between reported use of SP during pregnancy (from antenatal cards) and the risk of PM defined as any presence of asexual parasites or hemozoin pigment using placental histopathology. The proportion of women who reported taking 0, 1, 2 and 3 doses of SP during pregnancy was 6%, 36%, 57% and 2% women, respectively. The prevalence of placental malaria was 74% among women who reported taking 0-1 doses of SP and 60% among women who reported taking 2-3 doses of SP. Using multivariate logistic regression, women who reported taking 2-3 doses of SP had a lower odds of PM (OR=0.55, 95% CI 0.32-0.96, p=0.04) compared to those who reported taking 0-1 dose of SP. Compared to multigravida women, those who were secundagravida (OR=3.11, p=0.001) or primagravida (OR=6.13, p<0.001) had a higher odds of PM. Reported use of insecticide treated bed nets was associated with a lower odds of PM (OR=0.57, p=0.05) and women from households with a wealth index below the median had a higher odds of PM (OR=3.30, p<0.001). There was no association between the woman's education level and the odds of PM. The prevalence of placental malaria by histopathology was very high in an area of Uganda with high transmission intensity. The reported use of 2-3 doses of SP was associated with modest protection against PM despite widespread antifolte resistance which has been previously reported from this area.

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MATING-INDUCED GENE EXPRESSION CHANGES IN THE SPERMATHECA OF *ANOPHELES GAMBIAE*

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The high reproductive success of the malarial vector mosquito *Anopheles gambiae* is ensured by a single mating event; females store sperm from the male in a sperm storage organ called the spermatheca, using them to fertilize eggs which they develop after each blood meal. Sperm transferred during mating must be stored reliably to protect sperm quality from stresses encountered in the female such as free radicals produced during blood meal digestion. As *Anopheles* females mate only once and sperm are not replenished, mechanisms ensuring sperm viability must be functional for the lifetime of the female. Targeting sperm storage mechanisms in the female therefore would provide an alternative control strategy for reducing vector numbers and hence contribute to malaria control. As a step towards understanding the molecular mechanisms of sperm storage in female mosquitoes, we compared gene expression profiles of spermathecae 24 hours post-mating to age-matched virgins by genome-wide microarrays. We detected over 200 genes significantly up- or down-regulated in the sperm storage organs by mating. Mating-induced transcriptional changes were validated by qRT-PCR on a subset of genes in independent samples. Functional analysis of gene ontology terms showed up-regulated genes were involved in ion and sugar transport, suggesting a requirement for a specific nutrient environment within the spermatheca. Both up- and down-regulated genes contained annotations for diverse metabolic processes, in particular, protease metabolism. In addition, up-regulated genes also included components of several signalling pathways, suggesting signalling between the spermathecal contents and the surrounding cells. To investigate these signals further, we analysed the lower reproductive tracts of females mated to males lacking functional testes (and producing no sperm) and found that these expression changes did not require sperm. We hypothesise that additional small molecules from the male, either produced by the male accessory glands, or processed in the spermatheca itself, could trigger gene expression changes to preserve sperm quality. Functional analysis of candidate genes using RNA interference is highlighting mechanisms required for long-term sperm viability. These candidates could be targeted in the field to reduce the fertility of natural mosquito populations.

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INFLUENCES OF MALE SEMINAL FLUID PROTEINS IN THE ABSENCE OF SPERM ON FEMALE BLOOD FEEDING AND SEXUAL RECEPTIVITY FOR THE DENGUE VECTORS, *AEDES AEGYPTI* AND *AE. ALBOPICTUS*

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As part of a larger study on the function of male seminal fluid proteins (SFP) in *Aedes* mosquitoes, we investigated the effect of SFPs in combination with and without sperm on female blood feeding behavior and life history traits for *Ae. aegypti* (human blood meal volume ingested, digestion rate, feeding frequency, egg production and survival). In addition, we determined the dose-dependent effect of SFPs in the absence of sperm on female mating behavior in both *Ae. aegypti* and *Ae. albopictus*. Injected SFP homogenates stimulated egg production and feeding frequency to the level of naturally mated females. SFPs did not affect blood meal volume ingested, digestion rate, or survival. In addition, female *Ae. aegypti* and *Ae. albopictus* demonstrated high sensitivity to SFPs from their own species, with the majority of females becoming refractory to mating at doses ≥ 0.03 male-equivalents. This effect persisted up to thirty-four days post-injection. These results will aid future work to characterize individual SFPs involved in post-mating effects for these two important mosquito vectors.

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HETEROCHROMATIN PATTERNS IN MITOTIC CHROMOSOMES OF SPECIES FROM THE *ANOPHELES GAMBIAE* COMPLEX

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The *Anopheles gambiae* complex is a cluster of seven morphologically indistinguishable species. These species have distinct ecological and physiological adaptations apart from difference in vector capacity. *An. gambiae*, the major African malaria vector, is undergoing incipient speciation into two molecular forms, M and S. These two forms exhibit distinct differences in larval habitat, ecology, oviposition and behavior in the presence of predators. Heterochromatin is a gene-poor repeat-rich part of the eukaryotic genome. It makes up about one fourth of the *An. gambiae* genome. Heterochromatin plays a vital role in several biological functions, including chromosome function and gene regulation. It is also associated with rapid evolution and speciation. Here we studied heterochromatin patterns in the *An. gambiae* complex to evaluate if possible differences in heterochromatin may provide a key to better understanding of adaptation and speciation. Mitotic preparations obtained from imaginal discs of larvae were stained with two fluorescent dyes, DAPI and YOYO-1. We found differences in the number and size of the X chromosome heterochromatin bands throughout the *An. gambiae* complex. The laboratory strains of species showed a gradual increase in the amount of heterochromatin from *An. arabiensis* to *An. quadriannulatus* to *An. gambiae* and to *An. merus*. Laboratory strains tested for the M form (Akron and SUA2La) had a proximal heterochromatin band when stained with DAPI. In contrast, the laboratory strains tested for the S form (Pimperena, Zanu and Kisumu) exhibited both proximal and distal heterochromatin bands. We conclude that sibling species of the *An. gambiae* complex have fixed differences in the pattern and amount of the X chromosome heterochromatin. Future work would determine if natural populations of these species exhibit fixed heterochromatin differences and could lead to identification of possible causative links between heterochromatin variations and ecological adaptations of malaria vectors.

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PHYSICAL MAP OF *AEDES AEGYPTI* GENOME

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Mosquitoes are vectors of numerous deadly human diseases. To facilitate the development of new strategies for vector control, the genomes of the three most dangerous species of mosquitoes had been sequenced in the last decade. Among these three species, the vector of dengue and yellow fever viruses, *Aedes aegypti*, has the largest genome with 1,310 Mb, which is hard to assemble and map to chromosomes. Here, we report the first band-based chromosomal map of the donovani genome. Idiograms for the mitotic chromosomes of Ae. aegypti were developed. Three chromosomes were subdivided into 23 regions and 94 subdivisions. Instead of previously used cell lines, which usually accumulate chromosomal rearrangements, our method utilized chromosomes from a live mosquito. Based on results of fluorescent in situ hybridization (FISH), a total of 500 BAC clones from the largest genomic supercontigs was assigned to the specific band onto idiograms. The BAC clone locations within the supercontigs were predicted by fingerprint analysis. BAC clones were directly labeled with Cy3 and Cy5 fluorescent dyes by nick-

translation. Unspecific hybridization was prevented by adding unlabeled repetitive DNA fractions to the probe. From all BAC clones 106 were carrying previously mapped major genetic markers. These BAC clones were additionally ordered within each band by multicolor FISH because of the importance to link their genomic locations to the genetic locations of quantitative trait loci (QTL) related to pathogen transmission. The current study placed ~50% of the Ae. aegypti genome to precise chromosomal positions and also combined cytogenetic, genetic and genome maps into one integrated physical map (iMap) of the yellow fever mosquito. Further application of this map will enhance the quality of the current genome assembly of Ae. aegypti and also will help to find the genomic locations of QTL that might be important targets for developing advanced genome-based strategies for vector/disease control.

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SEARCHING FOR THE CULICINE MALE DETERMINING LOCUS

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Culex and *Aedes* mosquitos that vector deadly arboviruses such as West Nile virus, yellow fever, and dengue do not have heteromorphic sex chromosomes. Rather, sex is thought to be determined by a dominant autosomal "male determining locus" (MDL). The exact location, sequence, and gene product of the MDL remain unknown. We show that *Culex pipiens pallens* is the result of asymmetrical hybridization between putative *Cx. pipiens* females and *Cx. quinquefasciatus* males, two species that although closely related have unique genetic signatures. Significantly, as a result of non-recombination around critical sex loci, Cx. p. pallens males exhibit a DNA fragment identical to that of Cx. quinquefasciatus, and thus mosquito gender can be identified at any life stage. We have optimized SNPs focused on the region of the genome where we have evidence the MDL is located. We will discuss the impact of hybridization in mosquito evolution and control.

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MITOCHONDRIAL GENOME SEQUENCES REVEAL DEEP DIVERGENCES WITHIN THE *ANOPHELES PUNCTULATUS* GROUP

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Members of the *Anopheles punctulatus* group (AP group) are the primary vectors of human malaria and lymphatic filariasis in Papua New Guinea. Given their public health importance it is critical that we understand the species diversity and evolutionary history of *Anopheles*, for example, to determine why only certain mosquito species can transmit malaria and other human diseases. Here, we present the complete DNA sequences of 13 mitochondrial genomes from 7 distinct species: 5 from AP sibling species and 2 from the *An. dirus* complex in Southeast Asia. We assembled four sequences directly from whole genome sequencing data, while the remaining 9 mitochondrial genomes were sequenced simultaneously on one lane of an Illumina Hiseq 2000 instrument (after individual adapter-based barcoding). Our phylogenetic reconstruction suggests that the ancestor of the AP group arrived in Papua New Guinea 20 to 40 million years ago. Our results also reveal a deep divergence between *An. punctulatus* s.s and the *An. farauti* clade occurring 26 to 33 million years ago. This deep divergence within the AP group is interesting, as humans did not arrive in Papua New Guinea until 50 thousand years ago. We hypothesize that many malaria-related traits, such as human blood preference or the ability to carry human parasites, occurred independently in each *An. punctulatus* sibling species, which provides an opportunity to map these traits using comparative genomic methods. This deep divergence among AP mosquitoes also suggests that gene flow between species is limited and, therefore, that insecticide resistance is unlikely to spread from one species to another but instead would have to occur independently in each species.

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COMPARATIVE PHYLOGEOGRAPHY OF MALARIA MOSQUITOES FROM THE SOUTHWEST PACIFIC: POPULATION STRUCTURE, MITOCHONDRIAL INTROGRESSION AND THE REPEATED EVOLUTION OF NON-HUMAN BITING BEHAVIOR

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Using extensive geographical sampling, we examined and compared the phylogenetic relationships, phylogeography, and population structure of malaria vectors *Anopheles farauti* and *An. hinesorum* as well as the non-human biting *Anopheles irenicus* throughout their ranges in the southwest Pacific using mitochondrial (mtDNA COI) and nuclear (ribosomal protein S9 and ribosomal DNA ITS2) loci. Phylogenetic analyses suggest that non-human biting behaviour has evolved repeatedly (in populations of *An. hinesorum* and in *An. irenicus*), coincident with independent colonizations of the Solomon Islands. Maximum likelihood and Bayesian phylogenetic analyses of nuclear loci also showed that the three species are monophyletic. However, putative introgression of *An. hinesorum* mtDNA onto a nuclear background of *An. farauti* was evident in populations from Queensland, Torres Strait and southern New Guinea. Haplotype networks and pair-wise F_{ST} values show that there is significant genetic structure within New Guinea and Australia in both *An. farauti* and *An. hinesorum*, consistent with a long-term history of low gene flow among populations. Since some of the species under investigation (as well as other closely related anopheline mosquitoes) transmit malaria in the region so this is a medically important finding with regards to potentially understanding the mechanisms behind human feeding behaviour.

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THE EVOLUTION OF VIRULENCE OF WEST NILE VIRUS IN A MOSQUITO VECTOR: IMPLICATIONS FOR ARBOVIRUS ADAPTATION AND EVOLUTION

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Alterations to life-history traits resulting from infection of mosquito vectors with arthropod-borne viruses (arboviruses) could have significant effects on both vectorial capacity and the potential for arbovirus evolution and adaptation. Although arbovirus-vector interactions are generally characterized as benign, virulence has at times been noted. Previous studies with West Nile virus (WNV) demonstrated decreased fecundity in *Culex. tarsalis* mosquitoes associated with WNV infection, yet no cost of infection in *Cx. pipiens* mosquitoes in terms of survival, fecundity, or blood-feeding behaviour. In order to evaluate the potential for trade-offs between viral and vector fitness, studies assessing life history traits in *Cx. pipiens* were repeated with a *Cx. pipiens* -adapted WNV (WNV MP20). WNV MP20 was obtained by 20 sequential passages in *Cx. pipiens* and previously shown to have acquired both increased replicative ability and infectivity in *Cx. pipiens*. Results of current studies demonstrate that *Cx. pipiens* females fed on a WNV MP20 containing bloodmeal display significantly decreased survival relative to both unexposed or wildtype (WT) WNV-exposed mosquitoes. Specifically, WNV MP20-exposed females survived on average 16.9 days post-feeding, while mean survival of WT WNV-exposed and unexposed mosquitoes was 21.9 and 23.8 days,

respectively. In addition, both bloodfeeding behaviour and fecundity were altered in WNV MP20 exposed *Cx. pipiens* such that early feeding and fecundity were maximized at a later cost. When laboratory-evaluated differences in the probability of survival, bloodfeeding rates, and vector competence are used to compare vectorial capacities of *Cx. pipiens* infected with WT or MP20 WNV, results demonstrate that, despite increased viral fitness, vectorial capacity is in fact lower for the more adapted strain, demonstrating the inability of this strain to outcompete WT WNV on the population level. Although trade-offs between pathogen fitness and transmission have been identified in many other host-pathogen systems, these studies demonstrate for the first time that arbovirus evolution and adaptation may be constrained by the coupling of viral fitness and virulence in the vector.

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WEST NILE VIRUS EVOLVES TOWARDS INCREASED AVIAN FITNESS IN CALIFORNIA

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The COAV997 strain of West Nile virus (WNV) isolated from Imperial County in July 2003 was the first detection of WNV in California. WNV subsequently spread throughout the state and has become endemic. To investigate the spatiotemporal phenotypic evolution of WNV in California, the fitness of sixteen WNV isolates from four ecologically different areas was evaluated competitively using an *in vivo* avian model. An infectious clone derived virus of COAV997 was genetically marked with five nucleotide mutations (COAV997mut) and competed against isolates made from mosquitoes collected along a S-N transect from Coachella Valley to Sacramento from late 2007 and from early, mid and late 2008. Isolates were mixed 1:1 with the COAV997mut and inoculated into House Finches. The outcome of competitive replication was determined by quantifying the number of RNA copies in the serum of viremic birds by specific qRT-PCR. Results indicated that isolates from Coachella Valley, an area geographically close to the origin of COAV997, have not changed markedly compared to strains from northern study sites that have undergone clear increases in replicative fitness. No coincidental gain in virulence (mortality) was observed with increased fitness, with the exception of a spring isolate collected prior to the 2008 Los Angeles outbreak. In conclusion, the phenotype of most California WNV isolates has evolved towards increased replicative fitness compared to the 2003 invading strain, but the extent of selective pressure may have varied due to geographical or perhaps vector competence differences.

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EFFECT OF AVIAN COMMUNITY COMPOSITION AND MOSQUITO FEEDING PREFERENCE ON WEST NILE VIRUS (WNV) TRANSMISSION IN URBAN ENVIRONMENTS

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Heterogeneity in both mosquito biting behavior and avian reservoir competence are two important factors in the enzootic transmission cycle of West Nile Virus (WNV). The feeding preference for certain avian species among mosquitoes belonging to Culex pipiens complex is well documented. In addition, results from laboratory and field studies suggest that certain avian species act as more competent reservoirs than others in terms of amplification and transmission of WNV. Consequently, avian community composition is a key factor in the persistence and spread of WNV in urban area. We developed a fine scale Individual Based Model (IBM) model for enzootic WNV transmission which simultaneously explores the roles of mosquito feeding preference in an avian community as well as variation in reservoir competence within that community. This modeling approach allowed us to investigate the effects of heterogeneity among individual birds on the transmission dynamics of WNV and the role of individual birds that act as super-spreaders. Interactions among vector and host were described using a network in which links between the nodes (mosquitoes and birds) represented feeding events. We defined three avian classes based on preferential feeding behavior of *Culex pipiens*: preferred, semi-preferred, and non-preferred hosts. We also defined two avian classes based on their reservoir competence: highly competent and semi-competent hosts. The heterogeneity attributed to each set of classes was set using results obtained by 1) blood meal analysis performed on engorged *Culex* mosquitoes and 2) WNV serology performed on wild passerines that were sampled in Atlanta, Georgia, United States. Different scenarios were also modeled using data extrapolated from studies performed in other urban areas in the United States. Both the basic reproductive number (R0) and the daily effective reproductive number (Rd) were calculated to determine the probability of having a WNV outbreak in the modeled avian community and to estimate the effect of avian community composition on the spread of disease (accounting for the population's reduced susceptibility during the course of an epidemic), respectively. Results suggest that avian community composition has a strong impact on the spread of WNV in the urban environment and help better understand the role of super spreader individuals in the transmission dynamics of WNV.

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SHIFT IN DYNAMICS IN EASTERN EQUINE ENCEPHALITIS VIRUS ACTIVITY IN CENTRAL NEW YORK

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Eastern equine encephalitis virus (EEEV;*Togaviridae*:*Alphavirus*) is a highly pathogenic mosquito-borne virus that produces severe or fatal encephalitis in 30-50% of infected humans and horses. Recently, EEEV has undergone a regime shift in dynamics in the northeastern US and Canada. For three decades (1970-2000) periodic activity was detected in central NY, specifically in counties surrounding Oneida Lake, with 1-2 years of activity in mosquitoes and other hosts, followed by 1-6 years with no detectable activity. However, since 2003, there have been 9 consecutive years with active EEEV transmission, and a significantly higher prevalence of infection than in previous outbreaks ($p = 0.009$). This string of 9 consecutive years is a highly unlikely event to occur by chance ($p = 0.007$ with 15 of 28 years with EEEV presence). The current epizootic coincides with the arrival of a novel genotype of EEEV, the invasion of WNV to the region, and occurs within the backdrop of long term trends in temperature and declines in key songbird populations. The first human case in 26 years occurred in Oswego County in 2009 in a 70yo male, then again in 2010 in a 77yo male, and in 2011 in a 4yo child. EEEV-infected neurologic deer were found in 2008 and 2009. Serosurveys of hunter killed deer $\leq$ 2yo also have proven useful in determining the range of EEEV activity with 9 EEEV positive deer out of 179 tested from central NY 2007-2009. In 2011, 46 Culiseta melanura pools were found positive (MIR 4.2); the previous 3 year average was 43 (MIR 4.1). Similarly, in 2011,11 horses died from EEEV, while the previous 3 year average was 7 equines. In addition, two neurologic dogs died in 2011. Phylogenetic analysis of isolates indicates the virus is periodically introduced, most likely by migratory birds, but also overwinters in the central NY focus. However, we identified a genetic change in the virus in 2007, the year preceding the first human cases

seen since 1983. In summary, although the level of EEEV activity does not appear to be increasing significantly in the enzootic focus in central NY, it appears to be extending its range and intensity outside this historic focus.

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CHOLUL VIRUS IS A NOVEL ORTHOBUNYAVIRUS REASSORTANT CREATED BETWEEN CACHE VALLEY AND POTOSI VIRUSES AND A CAUSE OF INFECTION OF HUMANS AND LIVESTOCK IN THE YUCATAN PENINSULA OF MEXICO

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We have discovered a novel orthobunyavirus reassortant in the Yucatan Peninsula of Mexico. This virus, tentatively been named Cholul virus (CHLV), acquired its small RNA segment from Cache Valley virus (CVV) and its medium and large RNA segments from Potosi virus. To determine the seroprevalence of CHLV and selected other orthobunyaviruses in livestock in the Yucatan Peninsula, a serosurvey was performed using sera from 255 domestic animals (182 horses, 31 sheep, 37 chickens, 5 turkeys). Sera were initially screened at a single dilution (1:20) by plaque reduction neutralization test (PRNT) using 6 orthobunyaviruses: CHLV, CVV, South River virus (SORV), Kairi virus (KRIV), Maguari virus (MAGV) and Wyeomyia virus (WYOV). If neutralizing antibodies were detected, the serum was further diluted and subsequent PRNTs were performed to determine the end-point titer. Of the 182 horses, 63 (34.6%) were seropositive for CHLV, 49 (26.9%) were seropositive for CVV, 1 (0.5%) was seropositive for SORV, 56 (30.8%) had antibodies to an undetermined orthobunyavirus and 13 (7.1%) were negative. Of the 31 sheep, 8 (25.8%) were seropositive for CHLV, 3 (9.7%) were seropositive for CVV, 4 (12.9%) were seropositive for SORV, 14 (45.2%) had antibodies to an undetermined orthobunyavirus and 2 (6.5%) were negative. Four (11%) chickens had antibodies to an undetermined orthobunyavirus and 1 (20%) turkey was seropositive for CHLV. To determine whether orthobunyaviruses are also responsible for human infections in the Yucatan Peninsula, sera from 823 febrile patients that reside in this region were examined at a 1:20 dilution by PRNT using CVV. A total of 146 (18%) individuals had antibodies that neutralized CVV. Fifty sera were further analyzed at multiple dilutions by PRNT using CHLV, CVV, SORV, KRIV, MAGV and WYOV. Six individuals were seropositive for CVV, 5 were seropositive for CHLV, 1 was seropositive for SORV and 38 had antibodies to an undetermined orthobunyavirus. In summary, we demonstrate that orthobunyaviruses are a common cause of infection of humans and livestock in the Yucatan Peninsula.

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ARBOVIRUS SURVEILLANCE AND VIRUS ISOLATIONS FROM BATS IN UGANDA, KENYA, AND THE DEMOCRATIC REPUBLIC OF THE CONGO

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Zoonotic and vector-borne pathogens have comprised a significant component of emerging human diseases in recent decades. Arboviruses including Rift Valley fever (RVFV), Yellow fever (YFV), West Nile (WNV), Chikungunya (CHIKV) and Zika viruses have been isolated or detected serologically from various East African bat species, however the role of bats in arbovirus transmission cycles is poorly understood. The aim of this study was to investigate the exposure history of bats in East Africa to arboviruses as well as attempt virus isolation from bat tissues. Blood, liver, and spleen samples were collected from 281 bats from Uganda, 449 bats from Kenya, and 239 bats from the Democratic Republic of the Congo during 2011-2012. Liver and/or spleen samples from each bat were mechanically homogenized in tissue culture media and virus isolation was performed on Vero cells. Virus isolates were identified by either RT-PCR using virus group-specific primers, or by immunofluorescence assay. When available, serum samples were tested for neutralizing antibodies against WNV, YFV, Dengue 2 (DENV-2) virus, CHIKV, O'nyong-nyong virus, Babanki virus and RVFV by plaque reduction neutralization test. Virus isolates include Entebbe bat virus (*Flaviviridae: Flavivirus*) from *Chaerephon pumila* from Uganda, and as yet unidentified viruses from *Hipposideros commersoni* and *Triaenops persicus* in Kenya. *Epomophorus labiatus* fruit bats from Uganda demonstrated neutralizing antibodies against Babanki virus (1/33, 3%), RVFV (3/33, 9%), and flaviviruses (2/33, 6%). Flavivirus neutralizing antibodies were also found in *C. pumila* and *Mops condylura* from Uganda. Serological and virological evidence suggest that multiple species of fruit and insectivorous bats from East Africa are exposed to and are incidental or potential amplification hosts for arboviruses.

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ASSOCIATION OF SANDFLY FEVER SICILIAN VIRUS WITH AN OUTBREAK OF ACUTE FEBRILLE ILLNESS IN ETHIOPIA

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Sandfly Sicilian fever virus (SFSV) is a phlebovirus (family Bunyaviridae) transmitted by bites from sandflies. It was previously detected in the Mediterranean region with peaks reported in the summer in endemic areas. Clinical signs and symptoms range from a short, self-limited febrile syndrome to encephalitis and hemorrhagic fever. This study investigated the occurrence of an extensive cluster of cases of acute febrile illness (AFI) in the Afar region, Ethiopia. The Ethiopian Health & Nutrition Research

Institute (EHNR) requested KEMRI/CDC assistance with testing samples for Dengue fever as a recognized outbreak of dengue was ongoing in a neighboring area of Somalia at the time. Twenty-nine serum samples were screened by real time PCR for Dengue virus and selected viral hemorrhagic fevers. Subsequently, total nucleic acid extracts from these specimens were sent to Washington University in St. Louis for pathogen discovery using high throughput sequencing using the Roche/454 Titanium platform. Onset of symptoms was on September 5th, 2011; with case-patients reporting to the health facility starting three days later. A total of 12,816 cases were detected; of these, 9107 (71%) were male. The disease was described as self-limiting with symptoms resolving within 3 days of onset. All 29 serum samples tested negative for Dengue fever, Yellow Fever, Rift Valley Fever, Ebola-Zaire, Chikungunya Fever, Marburg and Crimean-Congo Hemorrhagic Fever Viruses. Computational analysis of the generated sequences identified 17 (59%) samples positive for SFSV, 1 for hepatitis B, and 2 positive for hepatitis GBV-C. Of the cases, 53% were female; the mean age of all patients was 28 years (range, 9 - 55 years, and median 25 years). All SFSV patients reported fever with periorbital and frontal headache. Other symptoms reported included, back pain (94%), loss of appetite (71%), joint pains (65%), vomiting/nausea (24%) and cough (18%) with no sore throat. This study indicates the occurrence of Sandfly Sicilian fever virus in the Afar region of Ethiopia. The resolution of symptoms after 3 days is similar to previous reports of this disease. This is the first study associating SFSV with an outbreak of AFI in Ethiopia. Detection of SFSV in these specimens by high throughput sequencing underscores the power of unbiased metagenomic strategies for pathogen detection. Additional studies are needed to determine the prevalence of Sandfly fever in this geographic area.

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DEVELOPING AN ANTIMICROBIAL RESISTANCE STRATEGY FOR ENTERIC SURVEILLANCE ACTIVITIES WITHIN A NETWORK A GLOBAL PARTNERS

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Diarrheal illness poses a significant risk to deployed military personnel and can result in compromised operational readiness and effectiveness through lost work days and increased healthcare utilization. Through a network of global partners, the Armed Forces Health Surveillance Center's Division of Global Emerging Infections System (AFHSC-GEIS) conducts enteric infection surveillance in support of force health protection and global public health. Guidance for enteric surveillance priorities are provided by a steering committee consisting of experts and stakeholders within the military system. AFHSC-GEIS partners in Cambodia, Egypt, Kenya, Peru, Thailand and the US have focused enteric surveillance efforts in populations ranging from adult travelers and deployed US military to host country military and pediatric populations to gain a better understanding of diarrheal illness etiologies. With the growing concern of antimicrobial resistance in bacterial organisms, the AFHSC-GEIS Enteric Surveillance Steering Committee is developing an Antimicrobial Resistance Strategy (ARS). This strategy responds to the need for data to guide treatment of traveler's diarrhea, detect emerging antimicrobial resistance of global public health interest, and develop a multi-site platform to study molecular mechanisms of resistance. Given that treatment for diarrhea in a particular region may differ, the strategy identifies a matrix of 'bug-drug' combinations that all network partners should be using for antimicrobial susceptibility testing and lays out an approach for sampling and additional enteric antimicrobial resistance research priorities. This presentation summarizes the antimicrobial resistance priorities outlined in the ARS and the approach used by the partner network to generate descriptive data on spatial and temporal trends for antimicrobial drug susceptibility of important enteric bacterial pathogens.

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PILOTING INTEGRATED COMMUNITY CASE MANAGEMENT OF MALARIA, PNEUMONIA AND DIARRHEA IN PRIVATE SECTOR DRUG SHOPS IN UGANDA

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Integrated Community Case Management (iCCM) of malaria, pneumonia and diarrhoea is typically implemented by lay community volunteers. However, the majority of febrile children in Uganda first seek care from the private sector, particularly at drug shops. The objective is to assess the feasibility and effects on quality of care of introducing diagnostics (malaria Rapid Diagnostic Tests and Respiratory Timers) and promoting pediatric-dosage pre-packed drugs for acute febrile illnesses (malaria and pneumonia) and diarrhea in private sector drug shops, in order to contribute to rational use of drugs, child survival and inform the introduction of private sector subsidy schemes. This is a proof of concept study with pre-post measurement using intervention and control districts. Subsidized drugs - Artemisinin Combination Therapy (ACTs), Amoxycillin tablets and Oral Rehydration Salts with Zinc (ORS/Zinc) - as well as diagnostics - malaria RDTs and Respiratory Timers - were introduced into all registered drug shops (n=44) in the intervention district. The control district continued to provide ACTs through the drug shops as was previously done. Household surveys, exit interviews at drug shops, in-depth interviews with drug sellers and focus group discussions with child-caretakers were conducted both at baseline in May 2011 and end line, in May 2012. At baseline, of children sick within the last 2 weeks, 496 (53.1%) first sought treatment in the private sector vs. 154 (16.5%) in a government health facility. Only 15 (10.3%) febrile children treated at drug shops received appropriate treatment for malaria. Of children with both cough and fast breathing, 5 (15.6%) received amoxicillin although none for 5-7 days. Of children with diarrhea, 8 (14.3%) received oral rehydration salts and none received zinc tablets. Supervision results indicate high utilization, that parents bring their sick children to drug shops for diagnostic testing, and high satisfaction among parents as well as drug shop staff. Results of the end line will be presented.

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IMPACT OF PERFORMANCE-BASED FINANCING ON PRIMARY HEALTH CARE SERVICES IN HAITI

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To strengthen Haiti's primary health care (PHC), the country piloted performance-based financing (PBF) in 1999 and subsequently expanded the approach to most non-government organizations. Under PBF, these organizations receive incentives up to 10% of base (services) funding for key PHC coverage achievements. This study evaluates 1) the costs of PBF implementation and 2) the impact of PBF on PHC's service delivery in Haiti. We obtained quantities of key services from four departments for 217 health centers (15 with PBF and 202 without) for 2008 through 2010, and computed quarterly growth rates. We analyzed the results using a difference-in-differences approach in multiple regression analyses by examining the differences in the trend between incentivized and non-incentivized services between PBF and non-PBF facilities to partial out potential confounding factors. For interpretation, we also interviewed staff in four facilities. Whereas technical assistance (TA) added 39% to base costs of PHC, incentive payments, on average, added only 6%. TA alone increased the quantities of PHC services over three years by 35% (2.7%/quarter). However, TA plus incentives increased these amounts by 87%

over three years (5.7%/quarter) compared to facilities with neither input. The growth in volumes of services with incentives and TA was 52% higher than the increase in facilities with support alone. Thus, incentives more than doubled the growth of services, a significant improvement ($p<0.05$). Improvements related to use of PBF were particularly associated with the utilization of maternal child services. Interviews indicated beneficial impacts on quantity and quality of services through careful monitoring and found no adverse impacts. Incentives proved to be a relatively inexpensive, well accepted, and very effective complement to TA, suggesting that a small amount of money, strategically used, can substantially improve PHC. Haiti's experience indicates that incentives remain an effective tool to strengthen PHC after more than a decade of use.

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WHO PAYS FOR MALARIA TREATMENT IN GHANA IN THE ERA OF HEALTH INSURANCE POLICY?

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Health insurance was instituted in 2005 as national policy by the government of Ghana to replace the cash and carry system of health care payment. This major financing reform in Ghana is a pro-poor intervention aimed at meeting basic health care needs of Ghanaians, with exemptions for vulnerable groups like children under five, pregnant women, and the aged. In recent years the out-of-pocket payments in national health insurance accredited health delivery facilities is rising. The paper investigates payment mechanisms households seeking treatment for malaria in Ghana use. It also assesses the socio-economic differentials among those using health insurance as a cushion for health care costs. The study is a cross sectional cost-of-illness study under the INDEPTH Network Effectiveness Safety Studies which employed quantitative data from the Dodowa Health and Demographic Surveillance System (HDSS) from October 2009 to December 2011. A household member who had been treated of fever within the last two weeks was interviewed about their expenditure on the treatment and the mechanism used to pay for the treatment. A total of 540 household members who received malaria treatment within the past two weeks were interviewed. Over 76% of household members paid out-of-pocket for treatment they received whereas 22% used health insurance and the remaining paid through an employer. An average of $33 (¢50.5) was borrowed by some patients to meet the health care cost. A bivariate analysis indicated that the poorest households are 90% more likely to pay out-of-pocket than the least poor (67%) for seeking malaria treatment. The analysis also showed that only 5% of the poorest patients are likely to use health insurance whiles the least poor are likely to use 42% of time, their health Insurance to pay for treatment. Out-of-pocket payments for health care are still significant component of health care costs in Ghana despite the fact that the national health insurance is in operation. The poorest patients continue to suffer the burden of malaria treatment expenses and borrow to pay out-of-pocket for care.

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OPTIMIZING CONTINUITY OF TREATMENT DURING UNREST IN KENYA, UGANDA, AND IVORY COAST

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This study explores the impact of emergencies caused by political unrest and violence on HIV treatment services in three countries, and identifies best practices to prevent treatment interruptions during an emergency. This is essential given that 200 million people and 30% of sub-Saharan Africans live in a state of chronic, recurrent or episodic emergency (WHO 2006). Data were obtained from over 50 key informant interviews with program managers, government officials, clinicians, and patients in Kenya, Uganda and Ivory Coast. Semi-structured, in-depth interviews were conducted in person and via telephone. Site visits to programs and clinics which provided HIV treatment during recent emergencies were also conducted. Common themes were identified to generate lessons learned. None of the study countries had national contingency plans for HIV treatment during emergencies prior to recent episodes of unrest. Informants described predictable challenges: disruptions to communication, transportation, and supply chain networks, and strain on human resources. Strategies for optimizing treatment continuity in emergencies should be developed at the policy (e.g., creating and disseminating national contingency plans for ART access during emergencies and inclusion of guidance on ART treatment during emergencies within national treatment guidelines), program (e.g., providing facilities with buffer stock and developing tracking and referral systems), and patient (e.g., establishing support systems for those who are displaced, providing patients with copies of medical records and educating patients on forced treatment interruptions) levels. In addition, responders to complex emergencies must be aware of the needs of PLWHIV. Emergencies are unpredictable but planning may optimize continuity of HIV treatment. Development of interventions at the policy, program and patient level in advance may prevent HIV treatment interruption when emergencies arise.

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TRAINING HEALTH WORKERS TO IMPROVE QUALITY OF CARE: DEVELOPMENT OF A THEORY-BASED TRAINING PACKAGE IN PATIENT-CENTERED SERVICES AND HEALTH CENTER MANAGEMENT IN UGANDA

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Universal access to appropriate malaria case management is advocated by the World Health Organization and others to reduce malaria morbidity and mortality in low income settings. However, increasing access to services has proven challenging and the evidence base is poor. In eastern Uganda, care seekers are discouraged from attending public health facilities due poor health center management, frequent drug stock-outs, limited skills and motivation of health facility staff, and poor relationships between health workers and communities. A large cluster randomized trial, the PRIME study, is evaluating a multi-faceted health facility-based intervention to address these shortcomings in Tororo, Uganda. Field activities began in December 2010, and the intervention was rolled out in May-June 2011. Study follow-up will continue until April 2013. A central component of the PRIME intervention is a series of nine interactive training modules to strengthen health worker-patient interactions to be

more patient-centered and to improve health center management in line with a revised system for maintaining supplies of rapid diagnostic tests and artemether-lumefantrine. The design of such interventions is rarely presented, reflected in the poor evidence base available for program planning. The methods used to design the PRIME modules, consisting of empirical formative research in the local area, a review of evidence of other interventions, articulation of a theory-based behavior change model, and piloting of the training modules will be reviewed. The impact of these training modules on proximal outcomes at 10 health facilities randomly assigned to receive the intervention, compared to 10 assigned to continue standard care, will be presented including daily patient attendance data, availability and management of key malaria commodities, and patient satisfaction with the health facility visit.

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WHERE TO DELIVER? INTENTION VERSUS PRACTICE IN PREGNANT WOMEN IN SOUTHERN PROVINCE, ZAMBIA

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In Zambia, 94% of pregnant women have at least one antenatal clinic visit, 52% of deliveries occur at home (72% in rural areas), a skilled health worker attends 47% of deliveries, and the maternal mortality ratio is 591 deaths/100,000 live births. Recently, the Zambian Ministry of Health has promoted facility-based deliveries with the goals of lowering maternal mortality and improving birth outcomes. Pregnant women were recruited at 90 health centers (HCs) during routine antenatal care in Southern Province, Zambia to participate in a large neonatal survival trial (ZamCAT). Enrolled women were asked where they planned to deliver and why, and were interviewed 4 days post-delivery about the delivery and reasons for any change of location. The 9,816 respondents had mean (± SD) age of 25.8 ± 6.9 y, median of 3 pregnancies, and 41% had only primary education. When interviewed antepartum, 93% of respondents intended to deliver at a HC, 6% at home, and 1% had no plan. However, 63% delivered in HCs, 36% at home and 0.9% elsewhere. Of those who intended to deliver at a HC, only 66% actually did. In contrast, 87% of women that planned to deliver at home actually delivered there ($p<0.01$). Women who delivered at home were older (26.8 vs. 25.1 y, $p<0.001$), less educated ($p<0.001$), and had higher parity (3.0 vs. 2.3 pregnancies, $p<0.001$). Women who intended to deliver at home and subsequently delivered at a HC stated they needed a skilled attendant, or for the safety of the mother and baby. Fifty-five percent of deliveries were attended by a nurse/midwife, 21.6% by a family member, 17.4% by a traditional birth attendant, and 3.7% self-delivered. Reasons for home delivery included distance, finances, and family/societal pressures. 12.6% of women who delivered at home gave other reasons including short duration of labor, no transportation, and timing of labor. To increase HC-based deliveries in rural areas of Africa such as this study site in Zambia, efforts need to be made to understand sociocultural barriers and to reduce costs of and facilitate transport to the HC.

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NATURAL INFECTION OF *LUTZOMYIA EVANSI* (DIPTERA: PSYCHODIDAE) WITH *LEISHMANIA* (*VIANNIA*) SPP. IN NORTHERN COLOMBIA

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The most important mixed focus of cutaneous and visceral leishmaniasis in Colombia is located in Los Montes de María, a mountainous region of the northern departments of Bolívar and Sucre. Although the phlebotomine sand fly *Lutzomyia evansi* is recognized in this area as the vector of *Leishmania infantum*, etiological agent of visceral leishmaniasis, the vectors of the parasites responsible for the cutaneous form remain unknown, motivating the search for natural infections in phlebotomines from the Montes de María area of Sucre. Flagellates were sought under the microscope in the dissected guts of sand flies captured by Shannon trap as well as daytime collections from peridomiciliar resting sites. The guts of infected insects were used for parasite isolation in NNN culture medium and molecular characterization by sequencing of the internal transcript spacer (ITS1) region. Two specimens of *Lu. evansi* were found infected with flagellates among 1619 captured, corresponding to an infection rate of 0.12%. Parasite distribution within the guts of infected phlebotomines corresponded to that exhibited by *Leishmania* species of the subgenus *Viannia*. Nucleotide sequence analysis allowed flagellates found in *Lu. evansi* to be grouped with reference strains and isolates of *Le. (Viannia) braziliensis* of cutaneous leishmaniasis patients resident in Montes de María. The natural infections of *Lu. evansi* with parasites of the subgenus *Le. (Viannia)* constitutes the first biological and molecular evidence of vectorial competence in this species and its possible participation in maintaining the epidemiological cycle of cutaneous leishmaniasis in northern Colombia.

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PREVALENCE OF SCABIES IN FIJI: A NATIONAL STUDY

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The World Health Organization estimates that 300 million people worldwide are affected with scabies each year Scabies and skin sores are recognised by cinicians and public health practitioners in Fiji and other countries in the Pacific region as significant health problems, however there are few population based data documenting the prevalence of scabies and skin sores in Fiji. We conducted a national epidemiological cross-sectional study to assess the prevalence of scabies and skin sores in collaboration with the Fiji Ministry of Health. The study enrolled 13,294 participants across all age groups and ethnicities. A total of 96 sites, including villages, settlements and schools were selected. The sample was representative of the national population of Fiji, particularly in regards to ethnic and age groups in Fiji. The all-ages prevalence of scabies was 23.3%. The prevalence of scabies varied by age group; there was a peak in children aged 4-7 years (51.1.%), and the prevalence was also high in the children aged three years and younger (36.7%). However, no age group was free of scabies. Bacterial infection of scabies was common with 20.7 of patients with scabies having evidence of super-infection. In conclusion, this is the first national prevalence study of scabies worldwide that we are aware of.. Scabies is a very common skin disease in all age groups in Fiji, and is particularly common in young children. We believe that this is likely to be the case in other tropical nations in the Pacific region, affecting up to 50% in this age group. Our data indicate that scabies has been underestimated as a tropical disease. A comprehensive and well-coordinated scabies elimination program is urgently needed. A study to assess the efficacy of a mass drug administration program is planned, comparing oral and topical treatment regimens. We aim to find the most appropriate and cost-effective solution to justify the investment of time, manpower and money to reduce the prevalence of scabies in Fiji and other tropical countries where scabies is endemic.

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IMMUNOMODULATION AT THE TICK-HOST-VIRUS INTERFACE

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Emerging and re-emerging diseases transmitted by blood feeding arthropods are significant global public health problems. Ticks transmit the greatest variety of pathogenic spirochetes, rickettsiae and viruses of any blood feeding arthropod. Infectious agents transmitted by ticks are delivered to the vertebrate host together with saliva at the bite site. Tick salivary glands produce complex cocktails of bioactive molecules that facilitate blood feeding and pathogen transmission by modulating host hemostasis, pain/itch responses, wound healing, and both innate and adaptive immunity. In this study, we have characterized tick borne encephalitis virus (TBEV) infected tick induced changes in cutaneous immune response at the early stages of attachment/feeding by Ixodes ricinus adults. Our preliminary analysis reveals that ticks (tick saliva) create an inflammatory environment at the bite site during the first 12 hours of feeding. Genes involved in neutrophil recruitment and migration were observed to be upregulated. We are currently investigating the immune cell recruitment at the bite site during the first 12 hours, and possibly identify the primary target cells for TBEV infection. Our study will advance the understanding of the immunomodulation at the tick-host interface induced by tick saliva that facilitates TBEV transmission and dissemination.

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DEVELOPMENT OF A MOLECULAR TAXONOMIC KEY FOR THE IDENTIFICATION OF SCRUB TYPHUS VECTORS, MITES WITHIN THE GENUS *LEPTOTROMBIDIUM*

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Larval trombiculid mites (chiggers) are important vectors of scrub typhus within Thailand and much of Asia. Identification of mite species is extremely difficult and we have proposed to develop a molecular taxonomic-key for the precise identification of trombiculid mites using the Cytochrome oxidase subunit I (COI) gene, 16s rRNA and 12s rRNA of mitochondria. Our aim to develop mtDNA barcoding is to identify the pre-defined species of mites collected from field sites, focusing mostly on the reservoir mites for scrub typhus. The evolutionary relationship among chiggers collected from wild-caught rodents trapped from different parts of Thailand was analyzed from the full-length COI gene (amino acid and nucleotide sequences), 16S rRNA, and 12S rRNA sequences. The maximum likelihood (ML) and neighbor joining methods using MEGA5 program were used to analyze the evolutionary relationship among field chiggers and the reference sequences retrieved from GenBank database. The Phylogenetic trees constructed from 12S and 16S sequences were supported by high bootstrap values on each cluster. These two sequences would probably be more suitable for chigger species differentiation. However due to the paucity of available gene sequence some species of chigger cannot be identified. Comparison of multiple DNA sequences and morphological ID will enable us to adjust and develop more accurate/rapid molecular assays for chigger species identification in the near

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IGM ANTIBODY RESPONSE OF GUINEA PIGS TO SALIVARY PROTEINS OF *TRIATOMA INFESTANS*

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Salivary proteins of triatomines like of other hematophagous arthropods, injected into the host during feeding, elicit a humoral immune response in their host. This immune response may indicate a recent exposure to triatomine bites and can be a potential measure of transmission risk of Chagas disease. Therefore in this study, IgM antibody responses of guinea pigs to salivary proteins of *Triatoma infestans* were analysed. Eighteen guinea pigs were exposed to low numbers of nymphal (n=5) or adult T. infestans (n=5) of three different strains from Chile, Argentina and Bolivia over a period of 10 weeks. The animal sera were tested for their IgM-antibody reactivity to crude *T. infestans* saliva. In ELISA assays, IgM responses were already detected after the first week of exposure to bug bites and they increased only slightly over time during the long term exposure study of guinea pigs to triatomines. Overall, IgM antibody levels of guinea pigs were lower in comparison to IgG antibody levels of guinea pigs analyzed in previous studies. Guinea pig sera from the long term exposure study were also used to probe for salivary proteins of *T. infestans* on Western blots in order to analyze the development of IgM anti-saliva specific immune responses. Guinea pig sera recognised salivary proteins with strain and developmental stage specific variations. Despite these variations, a 35 kDa antigen was detected by sera of almost all challenged guinea pigs. This antigen was also recognized by IgG antibodies analyzed in previous studies and may be a candidate exposure marker to detect triatomine bites. Because different triatomine species are capable of replacing *T. infestans* after vector control measures, the cross reactivity of immune responses to salivary proteins of different triatomine species were examined.

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GENETIC AND PHYLOGENETIC ANALYSIS OF SOUTH KOREAN SACBROOD VIRUS ISOLATES INFECTED IN HONEY BEE (*APIS CERANA*)

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Climate change will lead to movements of honey bees of different species and races, bringing them into contact with pathogens with which they have never co-evolved. Sacbrood virus (SBV) is one of the most dangerous viruses infected in honey bee and causes the failure to pupate and death in larvae and adult bee. Genetic analysis of SBV infected honey bee (*Apis cerena*) sampling from five different provinces, South Korea in 2010 was firstly carried out based on three nucleotide sequences of a partial structural protein coding sequence and two non-structural protein coding sequences. Sequences amplified by three specific primer pairs were aligned and compared with reference sequences deposited in GenBank database. Sequence alignments revealed a low level of sequence variation among Korean isolates (with ≥98.6% nucleotide identity), regardless of the genome regions used and geographic origins of the strains. Multiple sequence comparisons indicated that the investigated Korean SBV isolates are genetically closely related to Chinese and other Asian strains. Especially, Korean SBV isolates showed the numbers of private nucleotides and amino acids, which were not ever observed in other published strains. Korean isolates and a Chinese isolate (assigned as AF469603) hosted by *A. cerena* and a Chinese strain (assigned as HM237361) hosted by *A. mellifera* showed the difference in nucleotide and deduced amino acid identities. It strongly suggests that the host-specificity could be occurred among SBV strains isolated from different hosts. Phylogenetic relatedness between compared sequences was analyzed by MEGA 4.1 software using

neighbor-joining (NJ) method with boot-strap value of 1,000 replicates. Obtained topologies were in agreement with previous studies, in which, distinct groups of SBV were formed by a group of UK and European genotypes and a group Asian genotypes comprising strains originated from China, India and Nepal. However, phylogeny based on partial protein structural coding sequence grouped all Korean SBV isolates infected *A. cerena* as separate cluster. Our finding suggested further study including the Korean SBV isolated from *A. mellifera* is needed.

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IDENTIFICATION OF BLOODMEALS OF *LUTZOMYIA EVANSI (DIPTERA: PSYCHODIDAE)* IN RURAL AND URBAN ENVIRONMENTS OF NORTHERN COLOMBIA

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The methodologies traditionally used to identify wild and domestic reservoirs of *Leishmania* may require arduous fieldwork and produce a considerable ecological impact on the study areas. Among the new research alternatives generated to overcome these limitations is the use of molecular biological techniques to determine bloodmeal sources of phlebotomine sand fly vectors of *Leishmania* spp., thus helping to identify the vertebrate reservoirs of the parasite without the need to sacrifice large numbers of animals. In the present study a 358-bp segment of the mitochondrial cytochrome b gene was sequenced to identify the blood meals of *Lutzomyia evansi*, recognized vector of *Leishmania* spp. in the north of Colombia. Phlebotomines were collected in the rural area of Los Palmitos and Coloso, and the urban area of Ovejas, in the Colombian department of Sucre, where both visceral and cutaneous leishmaniasis are endemic. Analysis of bloodmeal sources of *Lu. evansi* show that it feeds on at least 7 species of vertebrates, among which *Bos taurus, Equus asinus* and *Homo sapiens* represented 68,4% of the total. Among the less abundant hosts *Sus scrofa, Equus caballus, Gallus gallus* and *Proechimys guyanensis* together constituted 14% of bloodmeals, while undetermined samples made up the remaining 17,5%. Some of the species encountered have previously been identified as reservoirs of *Leishmania* spp. The epidemiological implications of these findings are discussed.

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MICROENCAPSULATION OF AGRO-PEST BIOCONTROL AGENTS TO ENHANCE GLOBAL FOOD SECURITY

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Food security and availability are often associated with overall health quality at local and regional levels. For example, scarcity of food as the result of pestilence can be detrimental to subsistence-level farmers and consumers in poverty stricken regions of sub-Saharan Africa. Current strategies for controlling agricultural pests rely heavily on chemical pesticides. Pesticide resistance, environmental toxicity and unintended health consequences, nonetheless, are emerging issues. Biologically-based pest control agents have garnered much attention - *Metarhizium anasopliae* is highly effective in killing desert locusts (*Schistocerca gregaria*); *Bacillus subtilis* is being used to treat the agropathogenic fungus *Verticillium dahliae* while *Pantoea agglomerans* is used to target the fire-blight causing *Erwinia amylovora.* However, all these bio-control agents suffer from a high sensitivity to UV light, necessitating repeated applications and increased expense. In this work, we propose to package these bio-control microorganisms within an alginate polymer matrix to increase their resistance to UVC. Encapsulated cells in both "wet" and "dry" alginate microspheres resulted in significantly greater survival after extended UV irradiation compared to free cells in suspension. The inclusion of a high-carbon dye further increased UV resistance under "wet" conditions but did not augment protection under dried conditions. The concentration of carbon dye (0.01% - 5% v/v) directly correlates with cell viability following UV irradiation. However, dye concentrations greater than 5% were found to negatively impact overall cell survival. Increasing dye concentration had no effect on either microsphere shape or size. To examine a novel spray formulation, microspheres were incorporated into a guar-based resin. This resin further enhanced UV resistance in the "wet" state but did not significantly alter UV resistance in the dry state. These results represent a significant breakthrough in the use and implementation of bio-control agents in agricultural pest control.

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ECONOMIC EVALUATION OF AREA-WIDE PEST MANAGEMENT PROGRAM TO CONTROL ASIAN TIGER MOSQUITO IN NEW JERSEY

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Area-wide pest management (AWPM) is recommended to control urban mosquitoes, such as *Aedes albopictus*, which limit outdoor activities. While several evaluations of effectiveness exist, information on costs is lacking. Economic evaluation of such a program is important to help inform policy makers and obtain appropriate resources. We conducted an economic evaluation for an AWPM in Mercer and Monmouth counties, New Jersey, as part of a controlled design (AWPM vs. control). The study analyzed financial documents and time allocated by staff implementing the programs in 2009 and 2010. Also, random samples of households in AWPM and control areas were surveyed each fall since 2008 by a combination of mailed, telephone and in-person interviews. Sample sizes in data available ranged from 311 in 2008 to 396 in 2010. Hours lost were differences between actual and potential hours of yard and porch activities in an average summer week if there had been no mosquitoes. Net estimated benefits of AWPM were based on a difference-in-difference analysis (between years and areas). Reductions in hours lost were valued based on respondents' willingness to pay (WTP) for a hypothetical extra hour free of mosquitoes that they could spend in yard or porch activities. The share of residents in AWPM areas reporting mosquitoes as a major nuisance decreased from 68.6% in 2008 to 46.0% in 2010. The net impact was an 11.6% reduction in mosquitoes nuisances (p=0.11). Numbers of hours lost per week due to mosquitoes in AWPM areas between the base year (2008) and the second intervention year (2010) declined (mean±SEM) by 1.88±4.19 hours/week in intervention areas compared to control areas, indicating potential program effectiveness (p=.32). This translated to 24.4 hours gained over the 13-week summer. Residents' WTP averaged $2/hour (range: $1-$3), indicating a monetary valuation per resident of $49/year (range: $24-$73). The average per capita expenditure on AWPM was $30/year. These give a net benefit per resident of $19/year (range: -$6-$43) and a benefit-cost ratio of 1.63 (range: 0.81-2.44). The program had favorable behavioral impact. The benefit-cost analysis of data to date suggests a positive net benefit of the AWPM from residents being able to enjoy more time on porches and yards.

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EFFECTIVENESS OF THE AREA-WIDE PEST MANAGEMENT PROGRAM TO CONTROL ASIAN TIGER MOSQUITO IN NEW JERSEY: EVIDENCE FROM A HOUSEHOLD SURVEY

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Households' behaviors can both mitigate and measure the spread of urban mosquitos. Beginning in 2009, a comprehensive area-wide pest management (AWPM) project to control *Aedes albopictus* was implemented in 4 areas in Monmouth and Mercer Counties, New Jersey. Including other activities, the project focused on increasing residents' awareness, knowledge, and mosquito control practices. Evaluating the impact of this component is important to guide future AWPM programs. We analyzed household surveys conducted in the baseline year, 2008 (310 households), and second intervention year, 2010 (396 households) in AWPM and control areas. We measured changes in hours and mitigation expenditures (*e.g.*, repellents) from 2008 to 2010 in AWPM areas and compared results to corresponding changes in control areas. The average proportion of potential outdoor hours lost due to mosquitoes in intervention areas decreased (±SEM) from 29.7%±2.6% in 2008 to 24.3%±2.1% in 2010. Findings showed a net improvement of 7.0%±4.3% or an additional 1.89±1.9 hours spent in porch or yard activities due to AWPM (p=0.10). The share of residents bothered by mosquitoes in AWPM areas decreased from 68.6% in 2008 to 46.0% in 2010, with a net reduction in mosquitoes' nuisance of 11.6% (p=0.11). The percentage of respondents who reported cleaning their gutters in the last 12 months increased from 21.2%±3.5% in 2008 to 49.8%±3.4% in 2010, with a favorable net impact of 9.8%±7.0% of AWPM (p=.08) . The AWPM had favorable net reduction of 7.0%±5.3 in the share of households storing tires (p=.09) and a highly significant net increase of 20.4%±7.5 in the percentage of households who correctly reported the maximum number of days allowed to remove standing water to avoid breeding mosquitoes (p<.001). Analyses through 2010 found no statistically significant impact on expenditures. Nevertheless, the project has been effective in reducing the nuisance caused by urban mosquitoes and had a favorable impact on knowledge and several yard and porch activities. Data for 2011, to be added, will provide longer term impacts.

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FILARIAL NEMATODE INFECTIONS IN *AMBLYOMMA AMERICANUM* TICK POPULATIONS IN FAIRFAX COUNTY, VIRGINIA

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Filarial nematodes are associated with a number of public health problems and cause significant morbidity and mortality throughout the world. Arthropods often serve as intermediate hosts for these nematodes and pass them to their definitive host, often resulting in disease. Mosquitoes and blackflies vector the most well known filarial nematode infections, but ticks have been known to carry veterinary filarial nematodes. Recently, we discovered *Amblyomma americanum* tick populations in Maryland infected with filarial nematodes. This discovery was the first of its kind in the Mid-Atlantic and warranted further investigation. Ticks were collected at a single site in Fairfax County, Virginia between May and September 2011 via drag method and CO_2 traps. Collected ticks were then sorted by species and screened for filarial nematodes using PCR methods. Filaria amplicons were sequenced and analyzed to determine their association with known species. A total of 10 of 1223 (0.82%) *A. americanum* were positive for filarial nematode infections. Phylogenetic analysis revealed close associations with nematodes of the genera *Monanema*, *Acanthocheilonema*, *Dirofilaria*, and *Onchocerca*, which have been associated with zoonotic infections, suggesting that the filarial nematodes in Fairfax County tick populations could potentially be pathogens to humans. This initial data provides evidence that filarial nematode infections in tick populations may be emerging in the Mid-Atlantic region and increased surveillance is warranted to better characterize the nature of these infections.

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VECTORSURV AND THE RASTER CALCULATOR: TWO RECENT ENHANCEMENTS TO VECTORMAP FOR ESTIMATING VECTOR-BORNE DISEASE RISK

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VectorMap (www.vectormap.org) is a free resource for mapping collection data and distribution models of arthropods (mosquitoes, ticks, sand flies, mites) and vertebrate reservoirs involved in disease transmission. Collection data are comprised of vouchered specimen information, and both unpublished and unpublished observation data, and are quality controlled for geographic and taxonomic information. Users can view the locations of past vector collections, the results of models that predict the geographic extent of individual vector species, and various disease distribution layers, all in a GIS-type setting. This presentation focuses on two enhancements to VectorMap that have recently been developed: the Raster Calculator (RAC) and VectorSurv. The RAC allows the quantification, for any area of interest, of the overlap of models predicting the extent of vectors, hosts, and disease, to generate an estimate of hazard and risk. The RAC approach emphasizes the interactions and independent contribution to disease risk of the vector, host, and pathogen species predicted present. VectorSurv is designed for online reporting of regular surveillance data and allows the user to understand when vector population densities are above average, by providing information-rich graphs of species seasonality and abundance. These resources are offered as a place for publishing vector collection data, and for exploring the nexus between geography and vector-borne disease transmission.

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NEW PHLEBOVIRUS RELATED TO SANDFLY FEVER NAPLES VIRUS ISOLATED FROM SANDFLIES COLLECTED IN TUNISIA AND ITS POTENTIAL IMPACT ON PUBLIC HEALTH

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Sandflies are widely distributed around the Mediterranean. Therefore, human populations in this area are exposed to sandfly-transmitted diseases, including those caused by phleboviruses. While there is substantial data in countries located in the the northern part of the Mediterranean basin, few data are available from North Africa. Sandflies were collected from the site of Utique from the governorate of Bizerte located in Northern Tunisia, a well-known site of visceral leishmaniasis, during the summers of 2008, 2009, and 2010. In 2008 and 2009 sandflies were captured and pooled by sex and species. *Phlebotomus perniciosus* is the most abundant sandfly species (75%). Thus species identification was abandoned in 2010 and sandflies were pooled by sex. Sandflies were tested for the presence of phleboviruses by PCR. Viral RNA corresponding to a novel virus closely related to Toscana virus was detected in pools of sandflies collected in 2008, 2009, and 2010. Virus isolation in Vero

cells was achieved. Genetic and phylogentic characterisation based on sequences in the three genomic segments showed that it was a novel virus distinct from other recognized members of sandfly fever Naples virus complex. This novel virus was provisionally named Punique virus and transmitted mainly by *P. perniciosus*. To study the impact of this virus in term of public health in Northern Tunisia, a sero-epidemiological study was performed by Virus Neutralization Test using Toscana virus for the screeing stage (dilution 1/10 to 1/80). Positive sera were then tested concomitantly against Toscana and Punique virus by using two-fold dilutions from 1/10 to 1/2560 to discriminate between the two viruses. The results of the screening step indicated that a large percentage of the population had antibodies capable to neutralize Toscana virus (40.7%: 516/1266). The second stage will allow us to determine whether Punique virus is able to infect humans.

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DIVERSITY, DISTRIBUTION, AND ABUNDANCE OF MEDICALLY IMPORTANT SAND FLIES *(DIPTERA:PSYCHODIDAE, PHLEBOTOMINAE)* IN THE SOUTHERN PERUVIAN AMAZON BASIN

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Lutzomyia spp. are known to transmit leishmaniasis and bartonellosis throughout South America; however, information about sand fly distribution, abundance and vector potential is limited. Moreover, construction of an interoceanic highway in South America could have had an effect on the diversity and distribution of medically important sand flies. Herein, we examine habitat perturbation impacts on sand fly diversity and abundance by means of a survey conducted in six sites located along the interoceanic highway in the southern Peruvian Amazon Basin: Iberia and La Novia (Tahuamanu, Madre de Dios); Florida Baja, Alto Libertad, and Mazuko (Tambopata, Madre de Dios); El Carmen (Carabaya, Puno). Study sites were divided into 200, 600 and 1000 m transects along both margins of the highway. Sand flies were collected using CDC light traps, Shannon traps and protected human baits. A total of 7,381 sand flies were identified to two genera (*Lutzomyia* and *Brumptomyia*), 9 sub-genera, and 49 species, with 1,550 (21%) males and 5,831 (79%) females. CDC light traps, Shannon traps and protected human baits captured 3,894 (53%), 3,461 (47%), and 26 (0.4%) sand flies, respectively. The Shannon-Weaver diversity index (H) was higher in collections with CDC light traps (H=1.23) and Shannon Traps (H=1.01) than with human bait (H=0.67). The six most abundant collected species are known to transmit leishmaniasis in other Amazon Basin countries, and considered potential vectors in Peru: *Lutzomyia carrerai* (20%), *Lu. davisi* (14%), *Lu. richardwardi* (13%), *Lu. yucumensis* (11%), *Lu. shawi* (9%) and *Lu. hirsuta* (3%). Sites in Tambopata had the highest species diversity in this study: Alto Libertad (H=1.1), Florida Baja (H= 0.9), and Mazuko (H=0.9). Interestingly, 600 m transects (H=1.09 right margin; H=1.11 left margin) and 1000 m transects (H=0.97 right margin; H=1.22 left margin) had a higher diversity index than 200 m transects (H=0.81 right margin; H=0.83 left margin). Potential vectors represented 90% of the sand fly species in the 200 m transect, and 78% and 86% in the 600 m and 1000 m transects, respectively. This study provides information about sand fly species diversity, distribution and abundance, and suggests a widespread distribution of sand fly species that are known leishmaniasis vectors in the Amazon Basin. Future studies will determine *Leishmania* infection rates of these captured sand flies to predict and prevent disease transmission potential.

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MUTATIONS OF THE *MEXT* GENE IN *PSEUDOMONAS AERUGINOSA* ISOLATES ARE NOT ASSOCIATED WITH MULTIDRUG-RESISTANCE

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Pseudomonas aeruginosa is characterized by its ability to use multiple mechanisms to become resistant to several antibiotics. The extrusion of antimicrobials mediated by active efflux pumps represents one of them, with *P. aeruginosa* having 11 different known efflux pump systems. Among these, MexEF-OprN which is quiescent in *wild-type* strains *in vitro,* is over expressed in *nfxC*-type mutants, conferring resistance to quinolones, trimethoprim, chloramphenicol and imipenem. The *mexEF-OprN* expression in *P. aeruginosa* typically results from mutations in the *mexT* gene (positive regulator of this specific operon) and is associated with multidrug-resistance (MDR). From August 2011 through February 2012, 52 clinical isolates of *P. aeruginosa* were collected from Daniel A. Carrion Hospital in Lima, Peru and tested for drug susceptibility (*nfxC* phenotype) through disk-diffusion methods per Clinical and Laboratory Standards Institute (CSLI) guidelines. The *mexT* gene was amplified by PCR and mutations within it were detected by comparing sequencing data according to the PAO1 *P. aeruginosa mexT* gene reference sequence from the NCBI (National Center for Biotechnology Information). 45 isolates (85.6%) showed MDR to 3 or more antibiotics. The *nfxC*-type phenotype was found in 44 isolates (84.6%). 49 of the 52 amplified DNA samples were correctly sequenced and all were shown to have 8 consecutive deletions starting in the 105th position. Moreover, 20 punctual mutations were found throughout the sequences. 18 were synonym-type mutations and 2 were non-synonyms. There were 14 combinations of mutations arranged in 14 different genotypes. 9 genotypes were present among 42 of the MDR isolates and 5 genotypes present among 7 of the susceptible isolates. In conclusion, consecutive deletions at the 105th position and noted punctual mutations within the *mexT* gene were present in both sensitive and MDR isolates and are not directly associated with multidrug resistance.

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IN VITRO EVALUATION OF TWO HERBAL FORMULATIONS AS ANTI-INFECTIOUS MEDICINAL AGENTS

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This study evaluated the *in vitro* antimicrobial activity of two herbal formulations (A and B) against standard (n=7) and clinical bacterial (n=16) isolates using the agar-well diffusion method. In addition, the possible *in vivo* toxic effects were studied using Sprague-Dawley rat and the following phytochemicals, alkaloid, flavonoids, polyuronides, reducing sugars, cyanogenic glycoside, saponins, terpenes, anthracenosides, phytosterols and phenols screened. Formulation-A, inhibited the growth of 67% of the Gram-positive bacteria used while only 7% of the Gram-negative bacteria was inhibited. In total, Formulation-A inhibited the growth of a total of 23.8% of the microbes used. Formulation-B, inhibited all the Gram-positive bacteria and 60% of the Gram-negative bacteria used. In total, it inhibited the growth of 62% of microbes used. In the MIC analysis, Formulation-A exhibited minimum inhibitory concentrations ranging from 0.5 to 16.0 mg/ml for the standard strains whilst the wild strains had MICs ranging from 4.0 to 32.0 mg/ml. In the case of the Formulation-B, the MICs ranged between 1.0 and 2.0 mg/ml for the standard strains while for

the wild strains it ranged from 2.0 to 8.0 mg/ml. The study also revealed the presence of saponins, reducing sugars, phenolics, polyuronides, and triterpenes as the major phytochemical components of both formulations with alkaloids and flavonoids present only in the Formulation-B whilst phytosterols were only present in the Formulation-A. The LD_{50} value both formulations was greater than 5000 mg/kg, making both herbal medicinal products practically non-toxic. Thus, these formulations could be developed with further research into a potent antimicrobial.

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PREVALENCE OF RICKETTSIAL INFECTIONS AMONG FEVER PATIENTS IN HENAN PROVINCE, CHINA

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Rickettsioses are among the most important emerging and reemerging infectious diseases worldwide. Henan province, located in east-central China, is a newly determined endemic area for rickettsial diseases with limited knowledge as to the prevalence of the diseases and the identity and distribution of the rickettsial pathogens. A total of 291 serum samples were collected anonymously from patients presenting with acute febrile illness at the Fever Clinic of Zhengzhou University People's Hospital from August 2011 to March 2012. Whole cell antigens were used in enzyme-linked immunosorbent assays (ELISA) testing for specific antibodies (IgG) against spotted fever group (SFG), typhus group (TG) and scrub typhus group (STG) rickettsiae to determine the prevalence of exposure to rickettsial pathogens among febrile patients in Henan. 164 of 291 (56.4%) serum samples were collected from individuals who resided in the central areas of Henan Province in and surrounding Zhengzhou city, 66 (22.7%) were from eastern Henan, the rest 61 (21.0%) were from other areas. Overall 26.7% (52/195), 14.3% (26/182) and 4.7% (10/211) serum samples of the fever patients showed reactive antibodies against TG, SFG and STG rickettsiae, respectively. The exposure to SFG and STG rickettsial pathogens was less in urban (9.9% and 1.3%, respectively) but was more in rural areas (17.1% and 7.8%, respectively), while similar exposure levels were observed among fever patients from urban and rural areas to TG rickettsiae. TG and SFG rickettsial infections were found among fever patients from all locations in Henan Province, while seroreactivity to STG rickettsiae was only detected among individuals from eastern and south-eastern areas. The infection rates of male and female patients for TG and SFG were similar, while the infection rate for STG rickettsiae was higher in female patients (7.2%) than that in male patients (3.1%). This preliminary surveillance study revealed that rickettsial infections were not rare events among a fever patient population from Henan Province, east-central China. Physicians, residents and visitors should be aware of rickettsial diseases in this area. Future studies should include identification of specific rickettsial pathogens within the TG, SFG and STG responsible for the infections.

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INCREASED RESISTANCE TO AZITHROMYCIN IN *E. COLI* FOLLOWING MASS TREATMENT FOR TRACHOMA CONTROL IN RURAL TANZANIA

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Mass drug treatment with azithromycin (MDA) in trachoma endemic communities is part of the WHO-endorsed 'SAFE' strategy trachoma control programs. MDA has been shown to lower the prevalence of trachoma and lead to short-term reductions in other bacterial infections. However MDA can also lead to increased carriage of azithromycin resistance. In the context of a MDA program, we prospectively monitored azithromycin resistance in fecal *E. coli* collected from young children in 8 rural Tanzanian villages participating in the PRET+ Study. Four of the villages received MDA and 4 control villages did not. Rectal swabs were collected during cross-sectional surveys performed at baseline, 1, 3, and 6 months after MDA. *E. coli* isolated from the fecal specimens were screened for susceptibility to azithromycin using E-tests and to other antibiotics (ampicillin, amoxicillin-clavulanic acid, cotrimoxizole, ciprofloxacin, chloramphenicol, and erythromycin) using disk diffusion. The proportion of resistant isolates in the MDA and non-MDA villages was calculated for each survey; differences in proportion were compared using t tests. At the baseline survey the proportion of azithromycin resistant isolates was significantly greater in the non-MDA villages (0.19 vs 0.10, p=0.004). The proportion of azithromycin resistant isolates stayed roughly constant over the followup period whereas the increase in resistance was dramatic and sustained in the MDA villages (0.44 at the 1 month, 0.30 at 3 months and 0.22 at 6 months). The prevalence of resistance was statistically significantly higher in the MDA group at all follow-up surveys ($p<0.05$). In contrast, the prevalence of resistance to other non-macrolide antibiotics did not seem to be affected by MDA. Our study suggests that even a single dose of azithromycin results in significantly increased carriage of resistance over the 6 months following dosing and *E. coli* may be useful as a sentinel organism. While MDA is effective for trachoma elimination, it is not without costs; thus it is essential to monitor resistance levels in the wake of MDA.

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DEVELOPMENT OF ELISA FOR THE DETECTION OF *LEPTOSPIRA*-SPECIFIC ANTIBODIES USING RECOMBINANT ANTIGENS

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Leptospirosis is caused by spirochetes of the genus *Leptospira*. It is considered to be the most widespread zoonotic disease in the world. Symptoms of leptospirosis are easily confused with a variety of other febrile illnesses (e.g., dengue and malaria) that require different treatment regimens. Currently, the microscopic agglutination test (MAT) is the standard method for the diagnosis of leptospirosis. It is not only technically complex but also time-consuming. With the publication of the whole genome sequences of several pathogenic species of *Leptospira*, hundreds of genes encoding surface-exposed lipoproteins and outer membrane proteins were identified as candidates for the development of rapid diagnostics for leptospirosis. We chose three candidates (LipL32, LipL41, and LigA) and prepared recombinant proteins. MAT-confirmed positive sera from two geographic locations (Thailand and Latin America) were used to evaluate these antigens using ELISA. The results showed that with the combination of all three antigens, the overall sensitivity was 84%. Samples from infections due to a wide range of *Leptospira* serovars were reactive with these recombinant antigens in ELISA. These results suggest that an easy to perform ELISA with recombinant antigens for diagnosis of leptospirosis is achievable.

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CONFIRMATION OF *COXIELLA BURNETII* POSITIVE CLINICAL SPECIMENS: COMPARISON OF A SEQUENCING ASSAY AND A SECONDARY IS1111 REAL-TIME PCR ASSAY

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Coxiella burnetii, an obligate intracellular bacterium, is the causative agent of Q fever. *C. burnetii* is classified as a select agent because it is highly infectious, environmentally stable, and has been used as a biological

weapon. Acute Q fever is rarely fatal while chronic infections may have a case-fatality rate as high as 65%. Current methods for the detection of *C. burnetii* include direct, nested PCR and real-time PCR. Part of the multi-copy IS1111 element is currently used as a target for real-time PCR producing a 63-base-pair amplicon. The assay was modified to generate a larger product (208 bp) for confirmation of positive clinical samples through sequencing by designing a new reverse primer to complement the existing forward primer and probe for the IS1111 assay. Primer and probe concentrations were optimized using the 7500 Fast Dx (Life Technologies) instrument with PerfeCTa MultiPlex qPCR SuperMix (Quanta Biosciences). Ten banked positive samples were tested using the sequencing assay. Three additional primer and probe sets were designed to amplify the same multi-copy gene at various locations in the IS1111 element. The best performer was identified after optimization and used as a secondary assay for detection. The limit of detection for the assay is 1 fg DNA per reaction (~0.5 genome equivalents). Clinical specificity was assessed by testing 143 banked specimens. Real-time PCR results were in 100% agreement with banked positive DNAs and 98% agreement with banked negative DNAs. In conclusion, two sensitive and specific real-time assays have been developed that utilize the multi-copy IS1111 gene. One assay allows for DNA sequencing of the product and has been optimized to supplement real-time PCR for confirmation of positive clinical samples. A second real-time PCR assay was developed, optimized, and validated using banked specimens. The precision of the new assay is greater than the current PCR originally used to assess the clinical samples. Both assays may be used for the confirmation of *C. burnetii* in clinical samples.

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PREVIOUSLY UNCHARACTERIZED BIOLOGICAL ACTIVITY OF THE MACROLIDE TOXIN FROM *MYCOBACTERIUM ULCERANS*, MYCOLACTONE

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Mycobacterium ulcerans is the etiological agent causing the neglected tropical disease, Buruli ulcer. This disease is endemic to sub-Saharan Africa and Australia, with case reports primarily occurring in children. Clinically, the disease initially presents as a painless nodule, which deteriorates radially to a necrotic ulcer with characteristic undermined edges. There is a pronounced lack of inflammatory response at the site of ulcers, with only anecdotal mention of secondary infection in the literature. The macrolide toxin produced by *M. ulcerans*, mycolactone, is accepted as the sole virulence factor and has been shown to cause apoptosis and necrosis *in vivo*, as well as possess immunomodulatory properties. We hypothesized that the notable lack of secondary infection may also be a phenomenon mediated by mycolactone. Results in our laboratory have shown that exposure to mycolactone arrests the growth of specific bacterial strains, including *S. aureus* and *S. epidermidis*. Interestingly, there are no reports of clinically isolated mycolactone-deficient bacteria, and the common consensus is that selection pressure must be present to maintain the otherwise genetically unstable megaplasmid that encodes enzymes for mycolactone synthesis. Our results demonstrate an example of this selection. We have found that recovery of pMUM001 plasmid could be achieved simply by inclusion of lysates from arrested cell types. Given this data, we speculate that mycolactone is an "accidental toxin" whose intended function is to provide *M. ulcerans* a competitive advantage in its natural environment.

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DECISION-MAKING USING TRACHOMA SURVEY DATA IN KENYA: HOW MANY CLUSTERS ARE NEEDED FOR RELIABLE CONTROL DECISIONS?

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Mass drug administration (MDA) of azithromycin to trachoma endemic districts is a cornerstone of the SAFE strategy, which includes Surgery to correct trichiasis, Antibiotics to treat infection and Facial cleanliness and Environmental improvements to reduce transmission of the infective agent. Programs to eliminate blinding trachoma are complicated by changes in administrative boundaries prior to intervention, which may mean that previously collected survey data are not representative of new districts. The aim of this study was to explore whether existing data on trachomatous inflammation - follicular (TF) prevalence can be used to classify newly created districts and quantify the benefit from surveying further clusters. Existing TF prevalence data from 305 clusters in Kenya were used to examine the spatial characteristics of TF and conditionally simulate a 'gold standard' dataset which was used to evaluate a range of sampling scenarios. The performance of (i) existing survey data and (ii) the addition of further clusters were evaluated in terms of the ability to classify new districts according to WHO-defined MDA thresholds. Furthermore, the cost per correctly classified district was estimated with data from field surveys in Kenya and published estimates of treatment costs. Results showed that there was evidence of spatial clustering of disease risk across Kenya, which was partly attributable to large-scale climatic factors. Performance of existing data to correctly classify new districts was related to number of clusters surveyed and endemicity level, and negatively affected by proximity of district level prevalence to MDA prevalence thresholds. Increasing the number of clusters surveyed improved performance, with districts closer to thresholds requiring a greater number of clusters than low and high prevalence districts. This study suggests that the performance of existing TF data to classify newly created districts in Kenya is dependent both on the number of clusters and prevalence of TF, and thus the cost-efficiency of surveying additional sites will also vary with prevalence.

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PREVALENCE OF MATERNAL COLONIZATION IN DHAKA, BANGLADESH

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Insecticide thermal fogging (ITF) is a tool to control vector borne diseases. It is generally assumed that ITF reduces vector density independently of housing conditions. Few studies have been focused on Sand Flies while also looking at housing characteristics. We conducted a 15 month longitudinal study that included two deltamethrin based ITF interventions in 12 of the 24 houses at Comunidad de Trinidad Las Minas, Capira, Panamá, an hyperendemic cutaneous leishmaniasis transmission village. During the study we followed sand fly (SF) abundance. We found a 50 to 80% reduction in SF density at fogged houses when compared with control houses, while controlling for seasonal changes in SF abundance associated with rainfall. We found some heterogeneities in the reductions, as abundance changed according to SF species, with *Lutzomyia gomezi, L. panamensis, L. dysponeta* and *L. triramula* reducing their density between

40% and 90% after ITF, in contrast to *L. trapidoi* whose density increased 5% after the ITF. Differences in the impact of ITF were associated with housing quality, the most precarious houses, i.e., those with features that ease insect entrance, had a disproportionally larger SF abundance, in some cases with an increased domiciliary SF density following the ITF. Our results suggest that ITF success potential to control SF density and Leishmaniasis transmission could be dependent on housing quality.

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MULTIRESISTANCE IN CLINICAL ISOLATES OF *PSEUDOMONAS AERUGINOSA* FROM CUMANA, VENEZUELA

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Multidrug resistance in *Pseudomonas aeruginosa* (MDR-PA) is caused by the presence of intrinsic mechanisms and the acquisition of resistance genes from other bacteria. The presence of MDR-PA is of great concern because it limits the therapeutic options to treat patients. In this sense, we carried out a phenotypic evaluation of the resistance of 137 nosocomial strains of *P. aeruginosa* isolated from the general hospital of Cumana. These strains were classified according to standard biochemical tests and the antibiotic susceptibility was assessed using the Kirby-Bauer disk-diffusion assay, according to CLSI, using the antimicrobials: piperacillin, piperacillin/tazobactam, ceftazidime, cefepime, aztreonam, imipenem, meropenem, amikacine, gentamicin, tobramycin, netilmicin and ciprofloxacin. The results demonstrated that 64.5% (89/137) of the strains were resistant to at least one antibiotic. The strains were most resistant to CIP (51.1%), MEM (36.5%), IMP (35%), PIP (32.8%) and TZP (32.1%). Among the aminoglycosides, the strains were more resistant to NN (30.7%) and AN (29.9%). Of the 89 strains of *P. aeruginosa* resistant, 53 showed phenotypes that suggest the presence of mutations in the genes gyrA and parC, as well as 69 of them showed different phenotypes of resistance to beta-lactams, of which, 12 showed the presence of metallo-beta-lactamases. Results suggest that 49 strains had genes that code for aminoglycoside-modifying enzymes. Of the total of strains, 47 were MDR-PA. These results are very important to assess the presence different resistance mechanisms in the clinical strains of *P. aeruginosa* isolated and constitutes an alert for the high frequency of several mechanisms of resistance. Strategies most be designed and put in place in order to reduce the impact and the spread of these strains that can increase the morbidity and mortality in the patients and the costs for the health care system.

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DETECTION OF RESISTANCE GENES CODING FOR BETA-LACTAMASES TYPE-1, BLATEM, IN *ESCHERICHIA COLI* ISOLATED FROM RECREATIONAL BEACHES IN NORTHEASTERN VENEZUELA

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Escherichia coli can transfer antibiotic resistance genes to other strains of *E. coli*, as well as to other Enterobacteric species. Resistance of environmental strains of *E. coli* has been increasing and it has been suggested as a result of human waste contamination or agricultural related contamination. Here we assess the resistance of *E. coli* strains isolated from 4 recreational beaches in the Golfo de Cariaco, Sucre state and from 5 beaches in Margarita Island, Nueva Esparta state, Venezuela, for a period of 3 months, sampling every two weeks. We isolated and identified *E. coli* strains according standard biochemical tests and the antibacterial susceptibility was determined using the Kirby-Bauer disk-diffusion assay, according to CLSI. We detected blaSHV and blaTEM genes using PCR and the fragments of the amplified genes, as well as those of the 16S rRNA genes from the positive *E. coli* strains were sequenced. We isolated a total of 62 *E. coli* strains that were sensitive to amoxicillin-clavulanic acid, ampicillin-sulbactam, cefoxitin, ceftazidime, ceftriaxone, cefotaxime, cefepime, aztreonam, imipenem and meropenem. However, 23 strains were resistant to ampicillin and cefalotin, with intermediate resistance to piperacillin. All the strains showing broad-spectrum beta-lactamases did not amplified for blaSHV gene but amplified for blaTEM. Sequencing of these fragments showed that all of them were type 1 blaTEM. The sequences of the 16S rRNA showed that the strains that were isolated in the same beaches at different times were identical, but those strains isolated from different beaches showed nucleotide differences among them. This study demonstrates the presence of resistance genes coding for type-1 blaTEM in environmental strains of *E. coli*, whose origin can possibly be trace to human activities, which has implications for the transmission of these genes to the marine bacterial ecosystem.

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PYROSEQUENCING BASED ASSESSMENT OF THE BACTERIAL COMMUNITY STRUCTURE IN TICKS

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Ticks are important vectors for a variety of diseases. There is evidence that rickettsia may compete for the same niche within ticks. To better understand the tick's role as vector, we evaluated the microbiome of *Ixodes scapularis* ticks captured in Westchester, NY, and New Haven, Connecticut. Tick DNA was extracted and purified from 20 ticks, used to generate duplicate DNA amplicons of the V1-V3 hypervariable region of the bacterial 16S rDNA gene, and subjected to 454-pyrosequencing. On average, over 5000 sequences per sample were generated. The dominant taxonomic groups across all samples (0.1% of all sequences) were Acidobacteria, Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Firmicutes Spirochaetes, and Sphingobacteria. At the genus level, the dominant genera were *Anaplasma* (5.1% reads) *Borrelia* (3.1% reads), and *Rickettsia* (26% of reads). The majority of unclassified sequences belonged to class Enterobacteriales (26%). Also, members of the genus *Rickettsia* with highly divergent OTUs were detected. The abundance of genus *Rickettsia* was higher in female ticks (average 56% reads/tick) than male (average 12% reads/tick). Further, a total of 397 bacterial genera were present in our dataset and the median number of genera found per tick was 48 (IQR: 31-123). The median concordance of genera between replicates was 91% (IQR: 79-94%).

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ENVIRONMENTAL RISK FACTORS FOR RE-EMERGING EPIDEMIC TYPHUS

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Epidemic typhus is a rodent zoonosis transmitted to man by mucocutaneous or inhalational inoculation of causative rickettsia from infected lice or humans that continues to plague refugee populations. A new zoonotic reservoir of epidemic typhus in flying squirrels was

discovered in1975, and, since then, several cases of epidemic typhus and one of recrudescent typhus have been confirmed in patients without exposures to lice or infected humans. Forty cases of serologically or molecularly-confirmed *Rickettsia prowazekii*-caused epidemic typhus were collected over the period 1976 to 2007 and analyzed for environmental risk factors. Continuous variables were analyzed for significant differences by t-tests, and categorical variables were analyzed by chi-squares. Statistical significance was indicated by P-values ≤ 0.05. Most cases of epidemic typhus occurred east of the Mississippi River (P = 0.00001) and within the distribution range of the southern flying squirrel, *Glaucomys volans* (P = 0.00001). There were no statistically significant differences in the gender, age, or location of residence of the reported typhus cases; but significantly more cases occurred during the winter (P = 0.00001) than during other seasons. Case-patients were significantly more likely to report contact with flying squirrels or their nests, and to present with fever, headache, neurological manifestations, and maculopapular rash. There were no deaths. Sporadic epidemic typhus occurred in the eastern US, primarily during the winter in both rural and suburban/urban environments within the range of the southern flying squirrel and caused severe illness. Environmental health education and control strategies to reduce the likelihood of further clusters of epidemic typhus and to prevent recrudescent typhus in the eastern US should include modifying buildings and residences to prevent seasonal entry of flying squirrel colonies with their ectoparasites. The specific ectoparasitic vector(s) for flying squirrel-associated typhus has not been identified and will require further field investigations.

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MOLECULAR CHARACTERIZATION OF A NOVEL SCABIES MITE IMMUNE EVASION MECHANISM

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Scabies is a parasitic skin infestation caused by the mite *Sarcoptes scabiei*. Common worldwide, it remains a major public health problem in socially disadvantaged populations, including Australian indigenous communities. Scabies lesions are commonly co-infected with opportunistic bacteria, causing pyoderma. Scabies mites feed on epidermal protein, including host plasma; consequently, they are exposed to host defence mechanisms. We identified multiple scabies proteins functioning as inhibitors of the human complement system, a component of human innate immunity. Among them are a multi-gene family of proteolytically inactive serine protease paralogs (SMIPP-Ss), secreted into the mite intestinal tract and released into the epidermis. Our data indicate that SMIPP-Ss prevent complement-mediated damage of the mite gut. Two recombinant SMIPP-Ss investigated in detail exerted their inhibitory action due to binding of molecules involved in the three complement pathways. Immunohistochemistry demonstrated presence of the SMIPP-Ss in the mite gut, co-localising with serum components such as host IgG and complement. A neoepitope-specific antibody recognizing the pore forming surface-bound C5b-9 complex, an indicator of complement activation, did not exceed background levels, indicating that *in situ* complement activation does not occur the mite gut.We hypothesise that SMIPP-Ss facilitate mite survival and are attractive targets for the design of novel therapeutic agents. To understand the nature of the binding to complement factors, high resolution crystal structures (1.8 and 2.0 Å) of the two SMIPP-Ss were generated. Overlaying 30 SMIPP-S sequences on the two observed structures revealed small areas of high conservation representing possible exosites with potentially important function/s. Employing site-directed mutagenesis we are currently identifying the complement binding sites in SMIPP-Ss to determine the binding mechanism. This research may lead to the development of novel preventive and therapeutic strategies to control scabies and associated bacterial disease.

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RICKETTSIOSES IN HUMANS PRESENTING WITH FEBRILE ILLNESS IN FOUR SOUTH AMERICAN COUNTRIES

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Rickettsioses are caused by infection with intracellular rickettsiae transmitted to humans by arthropod vectors such as ticks. Although a handful of reports describe human disease in the countries of Brazil, Colombia, and Argentina, large scale serosurveys of febrile patients are lacking in the South American region. As part of a passive surveillance network for acute, undifferentiated febrile diseases, we performed serologic analysis (ELISA for IgG) for spotted fever group rickettsial (SFGR) and typhus group rickettsial (TGR) infection on samples obtained at an initial and convalescent visit in Peru, Bolivia, Ecuador, and Paraguay. Between 2007 and 2011, 8293 febrile individuals provided an acute and convalescent sample, and we identified 572 participants with at least a four-fold increase in titer to antibodies specific for rickettsia: 284 (49.7%) had a four-fold increase for antibodies against SFGR, 233 (40.7%) for TGR, and 55 (9.6%) for both groups of rickettsiae. No significant association was found between age or gender and incidence of rickettsial infection. The most frequent symptoms, regardless of the rickettsia group, were headache (94.8%), chills (92.5%), malaise (90.9%), and myalgia (89.2%). The proportion of febrile patients with serological evidence of SFGR and TGR was significantly higher in Peru (1.2% and 1.1%; p-values 0.002 and < 0.001, respectively) than in the other three countries. Because the largest number of positive samples came from the Amazon basin city of Iquitos, Peru, we chose this city to explore a possible association between incidence of infection and either season or climate. We found no correlation between season and infection incidence and also no correlation between precipitation and infection incidence.

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SCABIES - AN OUTBREAK IN THE OUTBACK OF AUSTRALIA

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Sarcoptes scabiei infections are uncommon in non-Indigenous Australians but are endemic in many remote Aboriginal and Torres Strait Islander communities in Northern Australia. Scabies underlies 50-70% of Group A streptococcal skin infections which are associated with Acute Post Streptococcal Glomerulonephritis and Acute Rheumatic Fever for which Indigenous Australians have the world's highest reported rates. Most scabies cases present with a classic infection of profuse pruritis and 5-10 mites burrowed in the skin, however a more severe refractory infection called crusted scabies manifests with thousands of mites that are highly transmissible from fomites as well as close personal contact. This study was undertaken to describe an outbreak of scabies in a remote Australian Aboriginal community in May 2011. An outbreak team was dispatched to an East Arnhem community to enhance the delivery of an ivermectin MDA after a suspected crusted scabies participant was identified in the community. The response team targeted the houses of identified household and classroom contacts in collaboration with the

local researchers who were implementing a population census for scabies and strongyloidiasis prevalence and an ivermectin MDA. Classical scabies infections were diagnosed clinically and crusted scabies from clinical and laboratory investigations. Participants were administered a stat dose of 200µg/kg ivermectin unless pregnant or their weight was <15kg. The alternative medications used were 10% crotamiton daily for 3 days or 1 application of 5% permethrin. Participants diagnosed with classical scabies received 2 treatments 2-3 weeks apart and those diagnosed with crusted scabies were referred to the local health centre for evacuation to the nearest hospital for more intensive treatment. One crusted scabies participant was identified clinically with 10 other school contacts who had classical scabies infections. There were 13 priority houses identified with 184 residents in total, a median of 14 (IQR 11-21) people per house. Of the 184 residents, 153 (83%) were screened and treated with 26(17%) having scabies lesions. In conclusion, the outbreak response took almost 2 months to complete. Community awareness of the increased scabies prevalence was high and treatment was sought after by individuals and families who were not all from the priority houses.

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CO-CIRCULATION OF MOSQUITO AND TICK-BORNE ARBOVIRUSES AMONG TICKS AND THEIR ANIMAL HOSTS IN THE PASTORAL ECO-ZONE OF IJARA DISTRICT, KENYA

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Tick-borne viruses have a significant impact on both human and animal health causing severe emerging and re-emerging infectious diseases in various parts of the world. Although we have evidence of circulation of mosquito-borne viruses in the pastoral zone of Ijara District, North Eastern Kenya, prevalence of tick-borne viruses and associated tick vectors remain unknown. This knowledge is important for planning response and control by the relevant authorities. This study aimed at determining the prevalence of tick-borne viruses circulating among ticks and/or their animal hosts in Ijara District, a pastoral zone and a major hotspot for mosquito-borne arboviruses in Kenya. A total of 1520 tick pools (10,488 ticks) were sampled from both wildlife and livestock hosts (cattle, goat, sheep, camel, lesser kudu, warthog, zebra and giraffe) at various sites in Ijara district. The sampled ticks were identified using morphological keys, processed for virus screening, isolation and identification using a combination of RT-PCR, isolation in Vero cells and sequencing of amplicons. The tick species sampled included: *Rh. pulchellus* (76.12%), *H. truncatum* (8.68%), *A. gemma* (5.00%), *A. lepidum* (4.34%), *H. marginatum* (2.24%), *Hyalloma spp* (0.92%), *Rh. Appendiculatus* (0.59%), *H. dromedarii* (0.59%), *B. annualtus* (0.53%), *A. hebraem* (0.39%), *Rh .pravus* (0.20), *D. rhinocerous* (0.07%) and unidentified nymph (0.20%). Bunyamwera, Ndumu, Semliki forest, Thogoto, Dugbe and West Nile viruses were isolated. Most of the detected viruses are known to be primarily mosquito-borne hence these findings constitute an important observation, suggesting the potential role of ticks in amplifying and disseminating viruses of public health importance far and wide. This is a first record of mosquito-borne Semliki forest, Bunyamwera and Ndumu virus isolation/detection in ticks. The observed co-circulation of viruses among ticks may provide opportunities for genetic changes and emergence of new arboviruses.

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ANIMAL HOST SKIN ODORS INCREASE TRAP CAPTURES OF ENGORGED RIFT VALLEY FEVER VECTORS IN HOTSPOT ZONES IN KENYA

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Learning cues that could reliably be associated with identifying a resource is a strategy employed by insects including mosquitoes in order to maximize the chances of obtaining the resource. These cues include host odors which would ensure that host-seeking mosquitoes locate their hosts for a blood meal. Here, we present data to suggest adaptive value of attractive host skin odors to engorged primary and secondary mosquito vectors of Rift Valley fever (RVF) based on field captures using skin odors of susceptible hosts (cow, donkey, goat, sheep and human) for this disease in two RVF hotspots in Kenya from April to September, 2011. Carbon dioxide traps baited with animal skin odors captured significantly more engorged RVF primary (*Aedes mcintoshi/ Ae. ochraceus/Ae. tricholabis/Ae. sudanensis*) (χ^2 = 60.96, df = 5, p = 0.04) and secondary vectors (*Mansonia* spp, *Culex* spp. and *Anopheles* spp.) (χ^2 = 212.7, df = 5, p = 0.002) than the control trap baited with CO_2 alone. Overall, engorged mosquitoes responses were in the order cow>goat>donkey>sheep>human. The findings suggest that, the inclusion of attractive skin odors to CO_2 baited traps can increase captures of engorged mosquito cohort and because of their previous host encounter, may offer the potential to improve sensitivity by increasing the likelihood of viral isolations.

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ANTIBODIES TO SPOTTED FEVER GROUP *RICKETTSIA* IN DOGS FROM URBAN SITES WITH REPORTS OF HUMAN ROCKY MOUNTAIN SPOTTED FEVER IN SAN JOSE, COSTA RICA

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In the past 5 years, at least 3 cases of Rocky Mountain spotted fever (RMSF) with no history of travel to endemic areas have been diagnosed in San Jose, Costa Rica. No animals or tick species were associated with these cases at the time of diagnosis. Dogs are common in urban environments and they may be implicated in transmission cycles of rickettsiae as victims and/or amplifying hosts. In this study, the possible role of dogs in the transmission of SFG rickettsia was evaluated at sites associated with human cases of RMSF. At each site, at least 50 dogs were identified within a radius of approximately 100 m from the house related to the human case, and blood samples were drawn. If the number of dogs was low, the radius was expanded until at least 50 samples were collected. Presence of IgG antibodies to SFG Rickettsia was evaluated by immunofluorescence assays using *Rickettsia rickettsii*, *R. amblyommii,* and *R. felis* antigen. Samples were considered positive when antibody titers were equal or greater than 1:32, and serial dilutions were performed to determine an end title in positive samples. In addition, ectoparasites from each dog were collected and analyzed by a PCR targeting a genus-specific fragment of the *Rickettsia* spp. citrate synthase gene (*gltA*). In one of the sites, serologic evidence of rickettsial infection was found in 8% of dogs (5/62), and end titles to *R. rickettsii* and *R. amblyommii* in three of them were 1:64 or greater. Antibodies to *R. felis* were also detected, although end titles were much lower than those of *R. amblyommii* and *R. rickettsii* in the same sample. Differentiation of these two species was not possible. One sample from a dog belonging to a human case diagnosed more than

two years prior to this study was positive with an end title of 1:2,048. In the areas evaluated, *Ctenocephalides felis* and *Rhipicephalus sanguineus* were the most common ectoparasites of dogs. Sequencing identified DNA of *R. felis* in fleas. These results demonstrate the occurrence of SFG rickettsia infection in dogs from urban sites in San Jose associated with human cases. Considering that *R. sanguineus* and *C. felis* are common and that they are capable of transmitting *R. rickettsii* and *R. felis* to humans, the possible role of dogs and their ectoparasites in the maintenance of these pathogenic rickettsiae in urban environments requires further investigation.

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SEVERE TUNGIASIS IN RURAL MADAGASCAR: REGRESSION OF MORBIDITY AFTER WEARING SHOES IN COMPARISON TO THE REGULAR APPLICATION OF A HERBAL REPELLENT

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Tungiasis (sand flea disease) is endemic in resource-poor communities in South America, the Caribbean and sub-Saharan Africa. Frequently, tungiasis is associated with important morbidity. In some patients the intensity of the infestation is so high, that feet are severely mutilated and the patient has difficulty in walking. During an intervention study in rural Madagascar, we identified eight individuals with severe tungiasis who were not eligible for the study, since the total number of lesions was impossible to be determined. These individuals were randomized into two groups: four received a repellent based on coconut oil and jojoba oil (the standard regimen for preventing new sand fleas to penetrate) twice daily on their feet. Four individuals received a pair of solid closed shoes. This was based on the rationale that tungiasis is a self-limiting skin disease and that shoes would protect against newly penetrating sand fleas. Over a period of 10 weeks, the participants were examined every two weeks and the severity score for acute tungiasis (SSAT- a score which encompasses all aspects of acute tungiasis-associated morbidity) and the severity score for chronic tungiasis (SSCT- a score for chronic tungiasis-associated morbidity) (Kehr et al. Parasitol Res 2007; 100:413-421) were measured. Besides, clinical pathology was documented with a digital camera equipped with a macro lens. During an observation period of 10 weeks, the SSAT decreased by 41% in the shoe group: median 19.5 (range 9-20) at baseline to 11.5 (5-16) at the end of the study. In the repellent group the SSAT decreased by 88%: median 17.5 (range 10-23) to 2 (2-4). In the shoe group the SSCT remained unchanged: median 8 (range 5-17) versus 8 (7-16), while it decreased slightly in the repellent group: median 8 (range 7-14) to 6.5 (5.5-14). Although the number of participants was very small, it can be concluded that the twice daily application of a plant-based repellent reduced extremely severe acute tungiasis-associated morbidity to an almost insignificant level. The donation of shoes also reduced severe acute morbidity, but the SSAT remained unacceptably high. We suppose that shoes, without wearing socks, only partially prevent the penetration of sand fleas.

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EXPOSURE OF OUTDOOR WORKERS IN NORTH CAROLINA TO TICK SPECIES AND TICK-BORNE INFECTIONS

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Forestry, parks, and wildlife workers have prolonged outdoor exposure, increasing their risk of being bitten by ticks and infection by tick-borne microbes. In 2011, we initiated a two-year investigation of the protection from tick bites provided by permethrin-impregnated clothing. Outdoor workers enrolled in our cohort study self-reported ticks bites and collected attached ticks. The ticks were identified to species and tested for common bacterial pathogens. The lone star tick (*Amblyommma americanum*), was the predominant species collected, accounting for 95% of the 429 ticks submitted. Other species collected included the Gulf Coast (*A. maculatum*), American dog (*Dermacentor variablis*) and black-legged ticks (*Ixodes scapularis*). Rickettsial organisms detected in ticks will be presented. Serologic tests of blood samples obtained at enrollment (n=127) revealed that many participants had pre-existing titers against spotted fever group rickettsiae. A minimum endpoint IFA titer of 1:128 was observed in 19% of participants against *Rickettsia rickettsii*, 23% against *R. parkeri*, and 11% against *R. amblyommii*. Fewer subjects had baseline titers against *E. chaffeensis* (4%). Comparison of titers from pre and post-season serum samples indicated that several participants seroconverted to spotted fever group rickettsiae and *Ehrlichia chaffeensis* during the course of the first year of follow-up.

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RECOMBINANT PROTEIN ANTIGENS DERIVED FROM OUTER MEMBRANE PROTEIN B OF *RICKETTSIA TYPHI* CAN BE USED AS DIAGNOSTIC REAGENTS

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Many different organisms are classified as rickettsial pathogens and they cause a variety of diseases. In recent years, emerging rickettsial diseases have been reported throughout the world and are a significant medical concern for local and deployed personnel and travelers. Symptoms of many rickettsial infections are easily confused with a variety of other pathogens (e.g., dengue, malaria, leptospirosis, etc.) that require different treatment regimens. In order to ensure that appropriate treatment is initiated promptly, the early diagnosis of rickettsial infections is critical. Currently, the diagnosis of rickettsial diseases relies mainly on serological methods and the immunofluoresecent indirect assay (IFA) has been the gold standard for diagnosis. These serological assays require the production of whole cell antigens in BSL-3 laboratories, which are not available in many endemic areas. Therefore, recombinant protein antigens, if shown to have similar sensitivity and specificity, can replace whole cell antigens as the diagnostic agents. The outer membrane protein B (OmpB) from *Rickettsia typhi* is a known immunodominant antigen and has been shown to have good sensitivity and specificity. In this report, two recombinant antigens that encompass the mature OmpB were generated by standard molecular biology techniques and evaluated for their utility in diagnosing IFA confirmed positive *R. typhi* infected patient sera. The ELISA-based assay using whole cell antigens, partially purified OmpB fraction and recombinant protein fragments showed 98%, 90% and 80% sensitivity when analyzing a total of 177 positive samples. The less sensitive assay offered by recombinant protein antigens may be due to the lack of lysine methylation as mature OmpB is known to have various lysine residues

methylated. The improvement of the recombinant protein antigens by enzymatic methylation is currently underway in order to better mimic the natural OmpB for higher sensitivity.

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SURVIVABILITY AND THE OPPORTUNITY FOR DISEASE TRANSMISSION: OBSERVATIONS OF THE *IXODES PACIFICUS*

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Ixodes pacificus the black-legged tick is the parasite responsible for transmitting *Borrelia burgdorferi* the bacterial agent of Lyme's disease. This apparently has become more prevalent with recent evidence of confirmed cases indicative of an increased incidence among the population in states such as Maryland, Pennsylvania, Wisconsin and Connecticut. Persons that appear to be predominantly affected include hikers, campers, residents in close proximity to wildlife areas and individuals that work in various professions including parks and natural resources personnel. Evidence suggests that conditions such as global climate change, human encroachment on wildlife habitat, and an increase in resident deer population in many locations also contribute to present favorable conditions implicated in *Ixodes* spp. dispersal. Here, we introduce the concept that the increased incidence in Lyme disease could be linked to the ability of the *Ixodes* species to survive extreme environmental conditions including reduced ambient air and blood meal. This was demonstrated through observation of ticks that were placed in an enclosed environment following removal from feeding source. Here, survival is described following removal from blood meal and ambient air for several days. However, introduction to minimal ambient air elicited visible Ixodes motility. This is possibly an indication of the species ability to survive under adverse conditions and certainly characteristics which might help explain the present increase in population in many communities. This phenomenon might also help to answer questions regarding a greater opportunity for infecting vulnerable hosts. This area needs further research to explain any possible genetic changes that might be aiding this survival ability, and possibly to offer further insight into time that vector and bacterium might be able to survive under those or similar extreme adverse conditions.

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THE TEMPORAL DEVELOPMENT OF ANTIBODY RESPONSES TO *SARCOPTES SCABIEI* INFECTION IN A PORCINE MODEL: RELEVANCE TO IMMUNODIAGNOSTICS

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No immunodiagnostic tests for human scabies are currently available, and existing animal tests are not sufficiently sensitive. The recombinant *Sarcoptes scabiei* antigen Sar s14.3 is a promising immunodiagnostic, eliciting high levels of IgE in infected patients. Limited data are available regarding the temporal development of antibodies to Sar s14.3, although this is relevant from a clinical perspective. We utilised a porcine model to measure scabies specific antibody levels by ELISA, comparing Sar s 14.3 to *S. scabiei* whole mite antigen extract (WMA). Robust IgG and IgE responses to both antigens were observed. Differences in the antibody profile between antigens were apparent, with Sar s 14.3 responses detected earlier and declining more rapidly after peak infestation compared to WMA. Both antigens resulted in >90% diagnostic sensitivity from weeks 8-16 post infection. These data provide important information on the temporal humoral immune responses in scabies and further supports the development of immunodiagnostic tests for scabies.

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IMPLEMENTATION OF AN INTEGRATED SURVEILLANCE SYSTEM IN RVF HOTSPOTS IN KENYA: ACHIEVEMENTS AND CHALLENGES

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In 2009, a consortium of partners were funded to establish an integrated surveillance system that incorporated public health, livestock and wildlife, vector and environmental sectors to monitor the activity of Rift Valley Fever (RVF) and other arboviruses in the RVF hotspots of Ijara in North Eastern (N.E.) and Baringo in the Rift Valley provinces of Kenya. Activities included sample collection from multiple host systems and species, laboratory analysis by taxonomic, serologic, tissue culture and molecular tools. Key features of the surveillance systems included the implementation of new-generation high throughput diagnostic tools. Delays in the implementation of the high throughput diagnostic platform presented challenges but use of conventional tools served as a fall back and provided level of successes in the surveillance system. Significant progress has been made in developing multiplexable arbovirus panels for sample analysis in the coming months. Virus activity detection/isolation; We have observed Inter-epidemic activity of RVF among the sentinel herds in the two hotspot areas in Rift Valley province and in N. E. Kenya (20% to 68% seroconversion rates respectively). However, despite seasonal upsurge of RVF virus primary and secondary vector populations in these locations, RVF virus activity in vectors was below detectable levels but, during the same period, other arbovirus species circulated actively among mosquitoes and ticks in detectable levels (73 and 51 virus positive pools respectively), with viruses like West Nile, Semliki Forest, Ndumu, Sindbis and Bunyamwera being detected. There has been no evidence of RVF infection/exposure among the wildlife species sampled. However, other arboviruses have been detected in ticks taken off some of the wild and domestic animals mainly alphaviruses, bunyamwera, dugbe (and dugbe-like), thogoto and unknowns, implying participation of livestock and wildlife in arbovirus maintenance and amplification in these areas. Serosurvey of sampled human populations demonstrated circulation of some of the viruses detected in vectors among humans possibly through transmission by vectors or exposure to infected animals. This underscores the value of setting up such integrated surveillance systems for arbovirus monitoring as it provides insights to dynamics of virus transmission and spread especially within a wildlife, livestock, human and vector interface.

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RICKETTSIA OF MILITARY IMPORTANCE: AN UPDATE

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Certainly the select agents *Rickettsia prowazekii*, *R. rickettsii* and *Coxiella burnetii* that cause epidemic typhus, Rocky Mountain spotted fever and Q fever are well known to military medical leaders and therefore are considered in their plans to protect the troops. However, currently the single most important rickettsial agent for military and civilian medical

leaders is Orientia tsutsugamushi, which causes approximately one million cases of scrub typhus a year within the Asia-Australia-Pacific region. The presence of this agent or closely related agents in UAE and Chile should increase the concern to military planners and world public health officials that scrub typhus is not limited to the previously described endemic area. Other new rickettsiae that should also be of concern to the DoD would be those that could have a significant impact on military missions such as *R. africae*, which in 1992 caused an outbreak of African tick-bite fever among US troops training in Botswana with an attack rate of approximately 30% and R. felis where 3.7% to 7.7% of fever patients in sub Saharan Africa caused flea-borne spotted fever. Thus, today not only do military medical leaders need to be concerned about previously described rickettsiae but also knew rickettsiae such *R. africae, R. felis, R. aeschlimannii, R.* spp. D-384, *R. raoultii*, and others.

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SEROEPIDEMIOLOGY OF SPOTTED FEVER GROUP *RICKETTSIAE* IN NORTH CAROLINA

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Worldwide, a large number of spotted fever group (SFG) rickettsiae have been recognized to cause disease in humans, while many more are considered to be nonpathogenic or of unknown pathogenicity to humans. In North Carolina, Rocky Mountain spotted-fever (RMSF), caused by *Rickettsia rickettsii*, is the most commonly reported tick-borne disease. Increasing entomologic and epidemiologic evidence suggests that other species of SFG rickettsiae may account for some of the reported cases of RMSF in the southeastern US. In order to better understand SFG rickettsiae infections in North Carolina, we conducted a retrospective seroepidemiologic study on paired serum samples submitted to the North Carolina State Laboratory of Public Health (NCSLPH) between 2008 and 2010. Criteria for inclusion in the study included patients for which there were paired sera available that had been submitted to the NCSLPH for testing against *R. rickettsii* and at least one of the sera had a titer greater than or equal to 1/64. We evaluated the serologic reactivity of the paired sera to *R. rickettsii*, *R. parkeri*, and *R. amblyommii* antigens. Of the 106 pairs tested, 22 were considered seroconversions (at least a four-fold rise or drop in titer) to at least one of the three antigens. Extensive cross-reactivity was found in 7 pairs, in which a seroconversion occurred against all three antigens. The number of seroconversions that were unique to each antigen were: one for *R. rickettsii*, four for *R. parkeri*, and six for *R. amblyommii*. Three pairs had seroconversions against both *R. parkeri* and *R. amblyommii,* but not *R. rickettsii*. Although serologic diagnostic methods for SFG rickettsiae are of limited value due to cross-reactivity, these findings are suggestive that species of SFG rickettsiae other than *R. rickettsii* are causing infections among North Carolina residents.

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A GLOBAL SYSTEMATIC REVIEW OF SCABIES AND PYODERMA PREVALENCE STUDIES

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The objective of this study was assess the global prevalence of scabies and pyoderma using surveys with uniform methodologies performed on the general population and representative of a wide geographical area, with a particular focus on developing countries. Three hundred and three surveys of the prevalence of scabies and pyoderma were found using a systematic review of MEDLINE and EMBASE from January 1985 to November 2011. Twenty-seven studies were selected for comparison based on their consistent methodological and diagnostic techniques. Only studies reporting population prevalence were included in the review. There is a high prevalence of scabies and pyoderma worldwide. The highest scabies rates recorded in the general population range between 33% and 46% in Panama and Ethiopia respectively. In each study, the scabies rates dramatically increased when only data on children were analysed. The highest scabies rates in studies involving children were recorded in Panama, with 78% of children under 2 years being affected. The Australian indigenous communities have also very high prevalence rates, reaching level between 32% and 35%. The prevalence of pyoderma recorded in these studies is also very high. In the general population, its highest prevalence ranged from 43% to 49% in the Solomon Islands and the Australian Aboriginal communities. Similarly to the trend of scabies rates, these studies clearly show that the rates of pyoderma increase in the younger populations. All studies were conducted in rural settings, except for two, where both urban and rural sites were selected and compared. In conclusion, this review includes a range of fairly diverse studies conducted around the world, but mostly in impoverished areas. Although the methods and settings may vary, the papers show very high rates of scabies and bacterial infections, particularly in children. In tropical developing countries, Sub-Saharan Africa, as well as the Australian Aboriginal communities, scabies and pyoderma appear to be highly prevalent. Although several studies have been conducted globally to assess the prevalence of scabies and pyoderma in community setting, no national study has been carried out to this date, other than the one conducted in Fiji by our research team.

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SPATIALLY EXPLICIT MEASURES OF SEASONALITY SHIFTS: NEW METHODS TO PROVIDE QUANTITATIVE ESTIMATES OF THE SHIFT VECTOR DISTRIBUTIONS UNDER ALTERED CLIMATES WITH AN APPLICATION TO LYME DISEASE

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Disease vectors respond to altered climate through changes in both population size and seasonality, and these dynamic characteristics contribute to transmission risk. Quantitative measures to analyze climate-driven shifts in population dynamics are lacking, particularly in the spatial domain. Traditional approaches, such as climate suitability indices, provide limited information on joint spatial-temporal changes in population. Here, we demonstrate the usefulness of spatially explicit mathematical modeling to address vector population response to climate change, and provide measures of projected spatial shifts in population size and seasonality for the Lyme disease (LD) vector. We assessed spatial changes in population dynamics of *Ixodes scapularis* for a baseline time period (2001-04) and two projected time period scenarios (2057-59), RCP 4.5 and 8.5, using a temperature-forced, multi-stage population model run across an eastern US grid of 4x4km cells. Multiple measures were developed to describe and compare the absolute size and timing of population signals across cells and between scenarios. Simulated populations under current climate were assessed against CDC observed LD incidence data. The projected response of *I. scapularis* to climate scenarios showed non-uniform geographic shifts in seasonality across life stages.Questing nymph and larval stages generally showed shifts to earlier questing seasons while questing was delayed in adults. Changes in season length were also observed in projected scenarios. *Peak population* size and *Peak month* (month of peak population) showed strongest associations in all life stages with CDC observed LD. The nymph exposure measure (*IP pop*), combining population size and timing, was a stronger predictor than *IP pop* in adult and larval stages. Methods presented reveal geographically varied response to climate in questing life stages, and some measures show agreement with observed data, suggesting their utility for examining climate-driven spatial shifts in population dynamics, seasonality and consequent vector-borne disease risk.

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CLINICAL RELEVANCE OF A DENGUE LYOPHILIZED ASSAY ON THE JOINT BIOLOGICAL AGENT IDENTIFICATION AND DIAGNOSTIC SYSTEM (JBAIDS) PLATFORM

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The Joint Biological Agent Identification and Diagnostic System (JBAIDS) is a ruggedized, real-time PCR-based system that is the United States Department of Defense's Program of Record for diagnostic testing of infectious diseases of operational concern. The Walter Reed Army Institute of Research (WRAIR), in collaboration with Idaho Technology, Inc. (ITI; Salt Lake City, UT), has developed a JBAIDS assay for the detection of dengue virus (the causative agent of dengue fever) in human serum specimens. A pilot clinical evaluation of JBAIDS Dengue Fever Detection Kit was conducted using a panel of 40 positive and 20 negative archived serum specimens (as determined by viral culture). These specimens, obtained from Navy Medical Research Unit-6 (NAMRU-6) in Lima, Peru, were collected in endemic areas from patients with a clinical suspicion of dengue fever. For this study, each specimen was divided and then processed concurrently and tested independently by two methods. The first aliquot of the specimen was extracted using the Qiagen RNA Viral Kit (Valencia, CA) followed by testing with a lyophilized universal dengue virus assay produced by Tetracore (Rockville, MD). The second aliquot of the specimen was processed using the ITI *1-2-3*™ Platinum Path Sample Purification Kit (ITI) followed by testing with the JBAIDS Dengue Fever Detection Kit (ITI). The Qiagen/Tetracore testing procedure was used as the independent validation assay. The Tetracore testing procedure validated all 40 positive specimens. Of the 20 negative specimens, one was found to be positive for dengue virus using the validation method and was excluded from further analysis. All of the validated dengue positive specimens were detected as positive using the JBAIDS Dengue Fever Detection Kit resulting in a positive percent agreement of 100% (40/40; 95% CI 91.2-100%). Of the 19 validated negative specimens, one was found to be positive using the JBAIDS Dengue Fever Detection Kit, resulting in a negative percent agreement of 94.7% (18/19; 95%CI 74.0-99.9%).

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PRECLINICAL EVALUATION OF VIRAL INTERFERENCE USING OPTIMIZED DENVAX-4 VACCINE VIRUSES

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DENV serotypes 1-4 cause dengue fever, dengue hemorrhagic fever, and dengue shock syndrome (DSS). The development of an effective vaccine represents an important approach to the prevention and control of this global emerging disease. Inviragen's tetravalent DEN vaccine (DENVax) consists of the live attenuated DEN-2 PDK-53 virus and three chimeras expressing the prM (premembrane) and E (envelope) structural proteins of DENV-1, DENV-3 and DENV-4 in the DEN-2 PDK-53 backbone (DENVax -1,- 3, and -4, respectively). We have demonstrated the safety and efficacy of DENVax in AG129 mice, monkeys, and humans. In these studies we have identified tetravalent formulations that induce neutralizing antibodies to all four DENV serotypes. However, the responses to DENVax-4 were limited by interference from the other, more robust chimeras. A common approach to overcome interference is to test different ratios of the four DENVax components. An alternative approach is to further optimize the DENVax-4 vaccine construct through genetic manipulation. We tested these hypotheses *in vitro* and *in vivo*. Specifically, we passaged the DENVax-4 vaccine strain in Vero cells, and identified adaptive mutations in the structural and nonstructural regions of DENVax-4. Additionally, we have reverted some of the mutations acquired by PDK-53 which do not contribute to vaccine safety and attenuation, but may impinge upon growth or replication of DENVax-4. These mutations were engineered to createseveral infectious clones. RNA transcribed *in vitro* from the infectious clones were used to transfect Vero cells. Ongoing characterization of the resulting DENVax variant strains includes increased viral replication *in vitro*, plaque size, and growth on mosquito cells. Additionally, we are evaluating the safety and immunogenicity of the DENVax variants in AG129 mice as mono- and tetravalent formulations. Immunogenicity of multivalent formulations will determine whether re-engineering vaccine strains and optimizing vaccine ratios can have an effect on viral interference in dengue vaccines.

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REAL-TIME QUANTITATIVE REVERSETRANSCRIPTASE-PCR (QRT-PCR) ASSAYS TO SUPPORT DENGUE VACCINE DEVELOPMENT AND CLINICAL CONFIRMATION OF WILD-TYPE DENGUE CASES

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The Sanofi Pasteur CYD dengue vaccine in Phase III efficacy trials is a tetravalent vaccine designed to elicit immune responses against the four wild-type dengue virus serotypes. qRT-PCR assays were developed and validated to assess dengue infections in subjects enrolled in clinical trials. To facilitate processing of the large number of samples, a screening assay targeting a dengue-conserved region of the 3'-UTR (Pan-DEN) was first used to identify potential positive sera, followed by four individual serotype assays. The serotype-specific assays each target conserved sequences of the dengue NS5 genomic region. Robustness, using a split-plot experimental design, assessed the impact of changes in PCR profiles, primer, probe and master mix concentrations. All assays were extremely robust, with less than 5-6% difference for most of the parameters tested, and <10% difference for two parameters. The assays were validated for specificity, precision (repeatability and intermediate), linearity/dilutability, matrix effects (lipidic and hemolytic sera and additional sera from non-dengue vaccine trials), range, accuracy, and two different automated nucleic acid extraction systems. All five assays are linear across their full range, are very precise (SD <0.3 log GEq/ml) even at the LLOQ (Lower Limit of Quantitation). All were highly specific, exhibiting <0.1 log GEq/ml absolute difference in expected values when competed against closely related targets. Matrix has little or no effect on expected values showing <0.3 log GEq/ml absolute difference in expected results when tested against hemolytic, lipidic, or homologous patient sera. The combined use of the Pan-DEN screening qRT-PCR assay with specific serotyping assays is a sensitive and specific test algorithm to identify and virologically confirm dengue cases.

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WICKING ASSAY FOR THE RAPID DETECTION OF DENGUE VIRAL ANTIGENS IN MOSQUITOES *(DIPTERA: CULICIDAE)*

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There is a real threat for dengue re-emergence in many regions of the United States especially in areas where the disease vectors, *Aedes aegypti*

and *Ae. Albopictus* are readily available. Unfortunately there are currently no accurate and reliable diagnostic methods to provide critical, real-time information for early detection of dengue virus within the vector populations in order to implement appropriate vector control and personal protective measures. In this paper, we report the ability of a novel lateral flow immunochromatographic assay developed by VecTOR Test Systems Inc. to determine if a pool of female *Aedes* mosquitoes is infected with any of the four dengue virus (DENV) serotypes. The DENV dipstick assay was simple to use, did not require a cold chain, and provided clear results within 30 minutes. It was highly specific and did not react with samples to which had been added Rift Valley fever virus, Chikungunya virus, Venezuelan Equine Encephalomyelitis virus, Japanese encephalitis virus, Ross River virus, LaCrosse virus, Caraparu virus, West Nile virus, and Yellow fever virus The DENV assay can provide rapid, easy to use assay to alert public health personnel to real-time critical information on the presence of DENV in mosquitoes. Results from this assay will allow a rapid threat assessment and the focusing of vector control measures at high-risk areas.

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ATTENUATED DISEASE IN POST-SECONDARY DENV-3 AND DENV-4 INFECTIONS IN IQUITOS, PERU

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Antibody induced by infection with any one of the four dengue virus serotypes (DENV 1-4) can influence the clinical outcome of subsequent infections with other serotypes, either enhancing or attenuating symptoms. While enhancement during second infections has been well studied, attenuation during third and fourth infections has not been rigorously examined. We estimated rates of disease as a function of DENV infection history using serological and surveillance data collected from longitudinal dengue studies in Iquitos, Peru, over 5 years. During this period, the city experienced intense transmission of DENV-3 (2006-2008) followed by DENV-4 (2008-2011). Infection history and DENV exposure during the study period were determined by PRNT70. Cases were detected by active door-to-door surveillance for acute febrile illness. Between September 2006 and February 2011, 39% (420/1077) and 53% (1595/2997) of the susceptible sample population seroconverted to DENV-3 and DENV-4, respectively. Clinical disease was detected in 7% (51/691) of DENV-3 infections and 10% (161/1595) of DENV-4. Rates of symptomatic illness among primary and secondary infections with DENV-3 (26% and 15%) were higher than in post-secondary infections (2%). Primary and secondary infections with DENV-4 also resulted in higher rates of disease (21% and 17%) than post-secondary infections (7%). Relative to individuals with <2 prior DENV exposures, odds ratios for risk of disease during post-secondary infections were 0.048 for DENV-3 and 0.22 for DENV-4. Post-secondary infections constituted a majority of seroconversions, and disease detected in these cases, despite attenuation, still represented a substantial proportion of all cases within the study population. Preexisting antibodies provided significant but incomplete protection against symptomatic illness during heterologous third and fourth infections. These findings have important implications for interpretation of surveillance data, estimating the global pattern of dengue transmission and burden of disease, and vaccine design and evaluation strategies.

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PHYLOGENETIC ANALYSIS OF DENGUE VIRUS TYPES 1 AND 4 CIRCULATING IN PUERTO RICO AND KEY WEST, FLORIDA DURING 2010 EPIDEMICS

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Dengue is caused by any of the four dengue virus types (DENV-1 to -4). DENV is primarily transmitted by the mosquito *Aedes aegypti* and most infections are asymptomatic or sub-clinical. In the US, dengue is endemic in Puerto Rico (PR), which in 2010 experienced the largest epidemic in its history with >21,000 dengue suspected cases, from which ≈75% were laboratory-confirmed. Autochthonous dengue transmission has occurred in Key West (KW), FL during 2009-2011. The aim of this study was to analyze the genetic makeup of DENV circulating among viremic blood donors from PR and KW during 2010 dengue epidemics. Plasma samples from 6 blood donors who tested repeated-reactive for DENV NS1 Ag in an investigational screening assay were used for further molecular testing by TaqMan qRT-PCR. Specimens were also tested for the presence of infectious virus by culture in C6/36 cells. Cell culture supernatants were tested for viral production by qRT-PCR, infectivity by focus-forming assay (FFA), and for sequencing of the envelope gene. Phylogenetic reconstructions were conducted by the Maximum Likelihood and Bayesian methods using MEGA5 and Mr. Bayes, respectively. All samples were confirmed positive for DENV RNA by qRT-PCR; 4 tested positive for DENV-1 and 2 for DENV-4. Plasma samples were infectious for C6/36 cells as determined by positive FFA and qRT-PCR results in culture supernatants. Analysis of the phylogenetic trees revealed that the three 2010 PR DENV-1 strains constitute a new lineage within genotype V, which are associated to other Caribbean and South American strains. The analyzed DENV-1 KW strain clustered with a strain isolated from mosquito pools collected in KW during 2010, and with Nicaraguan and Mexican strains. The two DENV-4 isolates obtained from PR associated with a number of strains that have circulated in the island during 1980s-1990s, indicating that this old lineage of the genotype II of DENV-4 still circulate in PR. This is the first report on the phylogeny of DENV circulating in PR and KW during 2010 epidemics.

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PARAMETERS ASSOCIATED WITH THE DEVELOPMENT OF DENGUE SEVERE DISEASE IN VENEZUELA

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Dengue is currently the fastest spreading viral vector-borne disease in the world. Although dengue viruses cause asymptomatic infections or mild disease (dengue fever (DF)), the most difficult to manage and feared forms are dengue hemorrhagic fever and dengue shock syndrome which can be fatal. To date, there are no vaccines or antiviral treatment modalities for dengue. Therefore, it becomes imperative to identify parameters that can be used by medical personnel to determine, early in the disease, which patients may be at a higher risk of evolving to severe illness, and allow early treatment intervention. Dengue in Maracay, Venezuela, is hyperendemic with co-circulation of the 4 viral serotypes. The increment of dengue transmission in Venezuela has coincided with an increase in the incidence of severe disease which in 2010 reached nearly 10% of all

cases. In this setting, a case-case study was set up in 2010 to compare clinical and laboratory parameters in patients presenting with DF versus patients developing severe dengue. Patients of all ages and presenting with fever and dengue clinical criteria to 3 designated health centres are recruited after written informed consent. Dengue infection and serotype is confirmed by RT-PCR. If positive, the patient is followed daily with clinical examination and sequential blood sampling at determined intervals up to 30 days. Severe cases are treated in a tertiary hospital and followed daily until discharge. Viral load dynamics, hematological parameters and serum levels of selected biochemical markers, including hepatic transaminases, cholesterol, triglycerids, panchreatic amylase are determined in blood samples obtained during the acute phase of the disease. Preliminary results point towards an association of severe disesase with lower levels of total cholesterol and higher levels of alkaline phosphatase and aspartate aminotransferase in this population.

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TEMPORAL DYNAMICS OF THE TRANSCRIPTIONAL RESPONSE TO DENGUE VIRUS INFECTION IN NICARAGUAN CHILDREN

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Infection with dengue virus (DENV) can result in a spectrum of disease from dengue fever (DF) to dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS). The clinical course evolves rapidly, and features of the host response associated with disease severity may be transient; differences in the response to primary (1°) and secondary (2°) DENV infection may also complicate efforts to identify predictors of disease severity. To characterize the temporal evolution of the host response in patients with 1°and 2° DENV infections, and identify features associated with clinical severity, we measured fever-day-specific genome-wide transcript abundance patterns in 105 peripheral blood mononuclear cell (PBMC) samples collected from 41 children hospitalized in Nicaragua, including 9 patients with 1° DF, 11 with 2° DF, 12 with DHF, and 9 with DSS; all but two of those with DHF/DSS had 2° DENV infections. We compared transcript abundance in patients to those in 8 healthy controls and observed that differences in patients with 1° DF were greatest on day 3, while changes in patients with 2° DF or DHF/DSS peaked on day 5 (FDR<1%). Prior DENV infection explained the greatest amount of variation in gene expression among patients and was associated with increased abundance of transcripts related to the mitotic cell cycle and B cell differentiation, and decreased abundance of transcripts associated with signal transduction and cell adhesion. This pattern was common to all patient groups, but was more pronounced and occurred earlier in patients with 2° DENV infection. There were also differences in transcript abundance associated with disease severity on day 3 that were not evident later: a set of interferon-stimulated transcripts was less abundant in patients who subsequently developed DSS compared to other patient groups ($p<0.05$, ranksum) and patients who developed DSS had higher levels of transcripts associated with mitochondrial function ($p<0.01$, ranksum). These findings demonstrate that differences in the timing and magnitude of a common host gene expression signature in DENV patients are related to both evidence of prior infection and disease severity, and highlight the dynamic nature of the early host response.

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EVALUATION OF THE DIAGNOSTIC PERFORMANCE OF THE CDC CLINICAL CASE DEFINITION FOR DENGUE, GUATEMALA: 2009-2011

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Dengue surveillance is a major priority in tropical countries due to the threat of devastating outbreaks. Diagnosis is typically based on the detection of acute febrile illness (AFI); however, suspected dengue cases are seldom confirmed with laboratory diagnosis. We evaluated the diagnostic performance of the CDC dengue case definition (CDC-D) among patients ≥2 years of age attending the Cuilapa National Hospital or Nueva Santa Rosa ambulatory facilities in the department of Santa Rosa, a low-elevation department in southern Guatemala, to differentiate dengue from other emergent diseases. The CDC-D requires fever and two or more of the following: retro-orbital pain, headache, rash, myalgia, arthralgia, leukopenia, and hemorrhagic manifestations. Active surveillance was conducted during 2009-2011 for AFI, defined as self-reported fever or measured temperature ≥38ºC that began <7 days before presentation with no other diagnosis (e.g. pneumonia or diarrhea). Leukocyte cell counts were unavailable for ambulatory cases, so we evaluated a modified CDC-D among those cases. Blood specimens were taken and tested for dengue virus by real-time polymerase chain reaction (qRT-PCR) and enzyme-linked immunosorbent assay for IgM. We defined as dengue-positive those cases with anti-dengue IgM positivity or dengue virus identification by qRT-PCR on acute blood samples. We evaluated 336 hospitalized cases, 52% of which were dengue-positive, and 84 ambulatory cases, 25% of which were dengue-positive. CDC-D in hospitalized cases had 92% sensitivity, 16% specificity, 58% positive predictive value, and diagnostic odds ratio (dOR) of 2.2 (95% CI:1.1-4.3) for dengue positivity. For ambulatory cases, the modified CDC-D had 88% sensitivity, 17% specificity, 31% positive predictive value, and dOR of 2.1 (95% CI:0.5.1-8.2). Dengue surveillance using the CDC clinical case definition would capture most dengue AFI but not exclude many dengue-negative AFI cases. Further research is needed to develop a more specific case definition.

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CLONING STRATEGY FOR THE 5´UTR-C GENOMIC REGION OF DENGUE 2 VIRUSES

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Dengue virus infections are one of the most important public health problems worldwide. The viral genome consists of a single stranded +RNA with an open reading frame (ORF) and two untranslated regions located at both 5´ (5´UTR) and 3´ (3´UTR) ends of the viral genome,

respectively. The 5´UTR and 3´UTR regions regulate viral replication and polypeptide translation initiation, which make them suitable for defining these fundamental mechanisms. The purpose of the present study was to develop a cloning strategy for inserting a 300 bp fragment coding for the 5´UTR, and the first 200 nt of the capside (5´UTR-C) of seven autochthonous and four foreign strains of dengue virus 2 (DENV-2), into the plasmid pTZ18R. The cloning strategy consisted of amplifying the DENV-2 5´UTR-C fragments by RT-PCR, linearizing the pTZ18R plasmid and ligating both molecules using T4 DNA ligase. Successful cloning of the DENV-2 5´UTR-C fragments was demonstrated by PCR of the transformed *E. coli* colonies and RFLP analysis with Bgl I. This is the first time this strategy has been used to clone the DENV-2 5´UTR-C gene segment. The resultant clones will be used to analyze the DENV-2 translation mechanism and its regulation with anti-sense molecules with potential inhibitory capacities.

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CHARACTERIZATION OF DENGUE VIRUSES OF ALTERNATIVE MORPHOLOGY

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Dengue Virus is one of the most important tropical diseases today, progressively spreading to every corner of the world. It is the causative agent for dengue fever, dengue hemorrhagic fever (DHF) and DHF/ dengue shock syndrome (DSS). Interestingly, classical dengue viral particles have never been visualized in acute patient plasma in spite of de facto high viremia. Our recent results, that virus can assemble into a mosaic of structures, or virus of alternative morphology (VAM), may explain the discrepancy. Immuno electron microscopy with patient plasma concentrates suggests that virus may be present inside of nondescript microvesicles (MVs). FACS data analysis has revealed that MVs from patient blood are predominantly derived from the platelets. Interestingly, dengue viral particles have been demonstrated inside of platelets isolated from acute dengue patients. Recent experiments demonstrate that infectious virus can be recovered from CD61+ cells with megakaryocytic characteristics in bone marrow of infected rhesus monkeys. Megakaryocytic cell lines, K562 and Meg01, therefore, were used as the model to further characterize VAMs. Supernatants from these infected cells are highly infectious, and yet, no classical virions have been observed. Fractionation studies by sucrose gradient revealed that high density (1.176-1.230g/ml), or heavy, microparticle rich fractions, had the most viral antigen, viral RNA, and were the most infectious in Vero cells by co-culture assays. Importantly, these VAMs were highly infectious in primary whole human bone marrow. The presence of VAM also suggests that humans likely acquire an alternative antibody response to counterattack them. Patient serum with high antibody titer to dengue viral E antigen was utilized to evaluate the neutralization capacity to the VAMs. A four-fold difference in neutralizing titer was observed between Vero-derived virus and K562-derived VAMs. This evidence suggests that focusing on the antibody response to megakaryocyte and/or platelet-derived virus may be a better index to predict protection from future infections.

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CHARACTERIZATION OF THE HUMORAL AND B CELL RESPONSE TO MONOVALENT AND TETRAVALENT DENGUE VACCINES

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Dengue virus (DENV) is the most common mosquito-borne viral disease worldwide, affecting 50-100 million people each year. There are four distinct serotypes of DENV, denoted DENV 1-4. Primary infection with any of these serotypes results in a debilitating illness that is generally not life-threatening; however, upon secondary infection with a heterotypic DENV serotype, risk increases for developing severe disease, characterized by vascular leakage leading to hypotensive shock, which may be fatal. A tetravalent (TV) live attenuated vaccine to protect against all four DENV serotypes has been developed by Inviragen (DENVax), together with monovalent vaccines (MV) against each serotype (DENVax 1-4). Previous mouse (AG129, interferon-α/β- & γ-receptor deficient) studies have shown the MV DENVax-4 vaccine to be less immunogenic than the other MV vaccines as well as in the TV vaccine. To further understand the humoral and B cell response to DENVax-4 and compare it with the TV formulation and wildtype (WT) DENV-4, B cell ELISpot assays against the four serotypes are being used to quantify DENV-specific antibody-secreting cells in spleen and bone marrow single-cell suspensions harvested from immunized A129 (interferon-α/β-receptor deficient) mice. Four groups and three time points are being analyzed: single immunization with DENVax TV or DENVax-4 or WT DENV-4 1036 and a control FTA diluent immunization, harvested on days 6, 14, and 28. *Ex vivo* B cell ELISpot analysis allows the quantification of DENV-specific plasma cells, while ELISpot using *in vitro* polyclonally stimulated cells enables the quantification of DENV-specific memory B cells. In addition, DENV-specific serum avidity against all four DENV serotypes is being measured using a modified ELISA assay with urea washes, and neutralizing antibody titers against the four serotypes is being determined using a plaque reduction neutralization assay. As a result of this study, we will be able to better characterize the humoral and B cell response to MV and TV vaccines and establish the basis for future vaccine studies using our improved mouse model of DENV infection and disease.

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SANOFI PASTEUR LIVE, ATTENUATED, TETRAVALENT DENGUE VACCINE TOXICITY, BIODISTRIBUTION AND SHEDDING STUDY IN THE CYNOMOLGUS MONKEY

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Sanofi Pasteur tetravalent dengue vaccine currently in Phase III clinical trial is a combination of four live, attenuated, recombinant viruses (CYD-1 to 4). Each CYD vaccine virus comprises genes encoding the non-structural proteins and capsid of the attenuated yellow fever vaccine virus, YF-17D, and the pre-membrane and envelope proteins of a wild-type dengue virus. As part of the nonclinical safety evaluation, the toxicity, biodistribution and shedding of CYD-1-4 was assessed in cynomolgus monkeys. Cynomolgus monkeys were given one subcutaneous injection of one human dose (5 $\log_{10}$ $CCID_{50}$/serotype) of CYD-1-4 or control, and were observed for 3, 9 or 21 days. Assessment included clinical observations, body temperature and weight, food consumption, clinical pathology, immunogenicity and post-mortem examinations including histopathology. Viral load, distribution, persistence and shedding of CYD-1-4 in tissues and body fluids were evaluated by qRT-PCR. The subcutaneous administration of the vaccine was well tolerated. All vaccinated animals seroconverted by day

21. Low level of CYD virus was detected in the blood and the virus was detected mainly at the injection site and the lymph nodes following the first 9 days after the injection. There were no toxicological findings other than the expected local reactions at the injection site, which correlates with previous toxicity data. These results, together with data from repeat-dose toxicity and neurovirulence studies, confirm there is no indication of toxicological concern, including risk of viscerotropism, with the CYD dengue vaccine and that transient distribution of the vaccine is limited mainly to the injection site and the lymphoid tissues.

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DENGUE AND INFLUENZA VIRUS CO-INFECTION INCREASES MORBIDITY, MORTALITY AND VIRAL LOAD IN WILD-TYPE MICE

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Both influenza and dengue are major public health problems worldwide. In 2009, Nicaragua experienced the largest dengue epidemic in over a decade, marked by atypical clinical presentation consisting of early onset of compensated shock and poor peripheral perfusion, as we observed in our two prospective studies of pediatric dengue in Managua. Multivariate analysis revealed only the year 2009 as a significant risk factor for this outcome, and neither DENV serotype nor clade changed. Our parallel influenza cohort study and national surveillance data showed that the pandemic of influenza A-H1N1 in 2009 overlapped for 8-10 weeks with the dengue epidemic. We hypothesized that previous or co-infection of influenza A-H1N1 and dengue virus may lead to altered immune responses and more severe disease, and initial testing indicated an increased risk of shock among children with anti-influenza A-H1N1-2009 antibodies, as measured by hemagglutination inhibition. Driven by this observation in humans, we decided to develop a mouse model to further explore the possible interaction of dengue and influenza virus infections. To do this, we established a mouse model of infection with mouse-adapted influenza A/PR/8/1934 (intranasal) and the virulent DENV2 strain D220 (intravenous) in wild-type C57/BL6 mice. In preliminary experiments, infection of both viruses within two days of each other caused increased morbidity as measured by weight loss, as well as lethality in 50% of the animals compared with sublethal infection of each virus alone. The lungs of co-infected animals displayed increased DENV titers as measured by quantitative real-time PCR. Influenza titers in the lungs are currently being quantified. In addition, we have expanded a clinical isolate of the 2009 pandemic H1N1 influenza A strain from Nicaragua, which we are currently testing in our influenza-dengue co-infection model. We plan to investigate the immunological pathway(s) involved in the interaction between the two viruses and test candidate genes using genetically deficient mice. This study will help identify targets for future therapeutic strategies for patients experiencing proximal infections of influenza and dengue viruses and may inform future vaccine strategies in endemic areas where both viruses regularly co-circulate.

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B CELL AND ANTIBODY REPERTOIRE IN SYMPTOMATIC PRIMARY AND SECONDARY HUMAN DENGUE VIRUS INFECTIONS

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The four serotypes of dengue virus (DENV) cause the most important arthropod-borne viral diseases of humans, dengue and severe dengue, with ten of millions of cases annually and 3 billion people worldwide at risk for infection. A secondary (2°) infection with a different DENV serotype is a risk factor for severe disease, but the mechanisms by which the host immune response to DENV provides either protection or enhancement of a subsequent infection with a different DENV serotype are poorly understood. We have leveraged our ongoing pediatric hospital-based and cohort studies of dengue in Managua, Nicaragua, to study the repertoire of B cells and antibodies (Abs) present during the acute phase of DENV2 primary (1°) and 2° infections by sorting single B cells from peripheral blood and generating recombinant monoclonal Abs (rmAbs) through PCR-based amplification of V(D)J gene rearrangements of Immunoglobulin (Ig) heavy ($_H$)- and light ($_L$)-chains. Among the first 8 peripheral blood mononuclear cell (PBMC) samples sorted from 2° dengue cases, we obtained ~3 plates of single PC-sorted cells (range 1-9 plates/sample), with a mean of 15-20 Ig V_H and V_L pairs/ plate. Data from the first patient samples analyzed indicated that 47% of recombinant antibodies were DENV-specific, as measured by ELISA to virions of the 4 DENV serotypes. Purified recombinant monoclonal antibodies are being produced and tested for their binding, neutralization and avidity capacities to the four DENV serotypes to generate a map of the frequencies, serotype-, antigen-, and epitope-specificity and functional profile. To this end, we have established a system using surface plasmon resonance (SPR) and purified DENV virions to measure avidity of recombinant mAbs and patient serum. The specificities of the rmAbs will then be correlated with immune status, overall DENV-specific neutralization and serum avidity. The potential impacts of these studies include increased understanding of the immune response to DENV infection, identification of potential therapeutic mAbs, and improved design of dengue vaccines.

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HIGHER SOCIO-ECONOMIC STATUS IS PROTECTIVE AGAINST DENGUE VIRUS INFECTION

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Dengue is a mosquito-borne illness that is a major public health problem in developing countries worldwide, particularly in urban settings. The Pediatric Dengue Cohort Study was established in 2004 to study the clinical spectrum and transmission of dengue in children in Managua, Nicaragua. In the first 6 years, annual participation ranged from 3,693 to 3,953 children aged 2 to 14 years. Children were enrolled prospectively, and data was systematically recorded on all medical visits. Participants are encouraged to come in at the first sign of illness and all medical

care is provided free through the study. Those with suspected dengue or undifferentiated fever are tested for dengue by RT-PCR, virus isolation, and serological assays. Additionally, yearly blood samples are collected and tested to determine the incidence of inapparent dengue virus infection. At enrollment, a basic demographic questionnaire was collected which included a number of socioeconomic indicators, and beginning in 2008 a more extensive socioeconomic status (SES) questionnaire was administered on a yearly basis. To examine the relationship between poverty and DENV infection and cases, univariate and multivariable generalized estimating equations (GEE) with a Poisson model were used. First principal component analysis was used to create a wealth index, and the index was then classified into tertiles (very poor, poor, and mid-SES). Variables included in the wealth index were: number of sleeping rooms, crowding, type of floor (dirt, concrete, ceramic/tile), type of housing construction (wood/plastic, concrete), access to water (inside house, outside house), and ownership of durable assets (cars, motorcycles, fans, televisions, refrigerators). Age was included in all multivariable models. In multivariable models, higher SES was a significant protective factor for DENV infection, with an incidence rate ratio (IRR) of 0.89 (95% CI 0.80, 0.98) for poor children when compared to very poor children and an IRR of 0.85 (95% CI 0.76, 0.96) for mid-SES children when compared to very poor children. Likewise, higher SES showed a trend towards being a protective factor for symptomatic disease (IRR 0.89; 95% CI 0.69, 1.14 for poor compared to very poor children and IRR 0.94, 95% CI 0.72, 1.24 for mid-SES compared to very poor children); however the results were not significant. These results support the concept that lower SES conditions are a risk factor for DENV infection.

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ECONOMIC COST AND DISEASE BURDEN OF DENGUE IN MEXICO: ADJUSTING FOR UNDER-REPORTING

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Despite eradication in the 1970s, epidemic dengue now affects 23 of 31 Mexican states, indicating a need to quantify the burden and cost of dengue to plan and assess control measures. In Mexico, dengue is reportable and case definitions have been promulgated. Protocols for laboratory confirmation include NS1, IgM and IgG tests at state and national levels. In 2011, 70,028 probable dengue cases and 50 dengue deaths were reported; however, official statistics often under-estimate the burden. Mexico, like 89 of 100 endemic countries, lacks cohort studies to ascertain under-reporting directly. Previous studies in the Americas have determined expansion factors (EFs) to estimate the true number of cases as multiples of the reported numbers. These EFs average 2.3 for hospitalized cases and 15.0 for ambulatory cases. We report here a study to quantify dengue burden and cost in Mexico. We are estimating the total number of cases in the public health system by 1) comparing medical records from selected hospitals with Ministry of Health surveillance data, 2) comparing case reports between 201 dengue monitoring facilities and other public facilities, and 3) using laboratory data on numbers of tests distributed, tests performed and positive cases (29.0% of 56,613 samples tested in 2011). We will extrapolate public sector data to the private health sector, initially by assuming a number of cases proportional to the relative sizes of the sectors. For example, the private sector has 35,000 beds and 14,000 doctors' offices, while the public sector has 79,000 hospital beds and 64,000 doctors' offices, giving ratios of 0.44 and 0.22, respectively. In addition to EFs, we will estimate 1) direct costs per dengue episode (adjusted by facility type), 2) indirect costs per dengue episode and due to death, and 3) disability-adjusted life-years burden based on literature review with WHO methodology. These procedures can improve estimates of dengue burden and cost both in Mexico and other countries in the region.

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DENGUE VIRUS PRM/E STRUCTURES REQUIRED FOR BUDDING AND INFECTION

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While the functions of Dengue virus (DENV) prM/E are well studied, most functional structures of the protein have only been partially mapped. Using Shotgun Mutagenesis technology, a comprehensive plasmid mutation library for DENV-3 prM/E was created in which each prM/E residue was individually mutated to a defined substitution, expressed in human cells, and analyzed for its effect on both viral budding and infectivity. To monitor budding, we used a virus particle production assay in which each prM/E mutant is expressed in a human cell line expressing the nonstructural genes required to produce DENV virus particles that can be detected in the media. To monitor infectivity, we used an infection assay in which each prM/E mutant is incorporated into infectious DENV reporter virus particles (RVPs) carrying a luciferase reporter that were used to infect permissive target cells. We also tested the contribution of each mutation to prM/E folding by measuring the reactivity of each mutant with conformation-dependent antibodies. In total, >1,100 mutants of DENV prM/E were individually tested. Critical residues comprising structures that contribute to each function were mapped onto the crystal structures for prM and Env in order to visualize and better understand how it functions.

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USE OF COMMERCIAL ELISA KITS IN DETERMINING THE PRESENCE OF DENGUE VIRUS NONSTRUCTURAL PROTEIN (NS1), ANTI-DENGUE IGG AND ANTI-DENGUE IGM IN HUMAN SERUM SAMPLES SUPPORTING DENGUE VACCINE DEVELOPMENT

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The CYD dengue vaccine is currently in phase III efficacy trials. Dengue cases in these trials are assessed by many measures including commercial kits for NS1 protein (BioRad) and anti-dengue IgM and IgG (Focus Diagnostics). The NS1 Ag ELISA is part of an algorithm to identify virologically confirmed dengue cases, the IgM and IgG ELISAs are used to serologically characterize dengue infections in an efficacy trial setting. The performance of these commercial kits in a clinical testing laboratory (CVD Mahidol) was assessed by determination of sensitivity, specificity, and serostatus agreement with an independent laboratory (Focus Diagnostics). Three independent assay runs for each commercial kit were executed, using sample panels of known NS1, anti-dengue IgG or anti dengue IgM serostatus. Of the results, 100% (90/90) of the NS1, 88.8% (80/90) of the anti-dengue IgG ELISA, and 98.8% (89/90) anti-dengue IgM results were correctly classified as positive or negative. The resulting sensitivity and specificity were both 100% for the NS1 ELISA, sensitivity was 93.3% and specificity was 84.4% for the anti-dengue IgG ELISA, and sensitivity was 100% and specificity was 97.8% for the anti-dengue IgM ELISA. The sensitivity and specificity determinations for the testing laboratory were within 10% of the independent laboratory for each of the three ELISAs indicating no evidence of a difference between laboratories. From these

data we conclude that these assays perform equivalently between the testing laboratory and an independent laboratory, and that specificity and sensitivity are acceptable for NS1 and anti-dengue IgM. The specificity of the anti-dengue IgG ELISA suggests that test data close to the threshold must be interpreted cautiously, as normal assay variability can result in identification of these samples as positive or negative. During efficacy trials, samples may be tested using all three commercial kits, these analytical tools will be informative for dengue case characterization.

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CLINICAL RULE TO PREDICT RECURRENT SHOCK IN DENGUE SHOCK SYNDROME

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Dengue has become a major public-health burden in tropical and subtropical areas of the world, mostly in South-East Asia and Western Pacific Regions. This disease ranges from asymptomatic or mild fever (DF) to severe dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Mortality in dengue infection is mostly due to the severe form of dengue infection especially the DSS. However, very few study described the recurrent shock that reportedly affects the protocol therapy. Here we aim to find the prevalence of recurrent shock and develop a prediction rule to identify these cases in the early stage of DSS. A prospective study was conducted in two hospitals in Vietnam to identify specific predictors of recurrent shock. Independent predictors were identified using uni-variate analysis. The data set was randomly split into two data sets including derivation and validation sets. The binary logistic regression was used to derive a scoring system for identification of recurrent shock using the derivation set. The performance of the prediction rule was evaluated by receiver operating characteristic (ROC) curve analysis of validation set. Our study included 444 DSS patients, 126 of whom had recurrent shock (28%). The uni-variate analysis showed that shorter admission day, subcutaneous bleeding, gastrointestinal bleeding, ascites, hemoconcentration, low platelet count, narrow pulse pressure were risk factors for development of recurrent shock. A prediction rule was developed based on ann equation of five variables including admission day, subcutaneous bleeding, ascites, blood platelet count, blood leukocytes, and pulse pressure. Our rule showed a relative high sensitivity at 68% and a moderate specificity at 59%, with area under the curve at 0.701. In conclusion, we have derived a clinical rule that could assist clinicians to predict the recurrent shock in the early stage of DSS that could assist clinicians to closely monitor the critical patients.

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OBSERVATIONS FROM TWO COMMUNITY LYMPHOEDEMA INITIATIVES IN INDIA AND ETHIOPIA

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Lymphoedema or elephantiasis is a common and devastating public health problem in many developing countries. Epidemiologically significant causes include infection for example filariasis or due to geodermatological interactions in the case of podoconiosis. Detailed examination by dermatological specialists have not been reported previously to assess the specific impact on the skin. Observation of patients attending lymphoedema community clinics in India and Ethiopia were undertaken in November 2010. Patients were examined by dermatologists in both settings and diagnoses recorded. Whilst in both settings the majority of cases were deemed clinically consistent with the main local cause, other causes of leg swelling and additional dermatoses were identified. This presentation details the findings of the diagnoses observed together with a brief description of the two community services in place for caring for this condition from which many millions of those living in resource poor settings remain untreated and uncared for. Lymphatic failure regardless of the underlying cause produces similar clinical features. Whatever the underlying cause for lymphoedema simple skin care intervention can have a dramatic effect on clinical appearance and severity of the disease as well as quality of life.

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FILARIAL POLYPARASITISM COMPLICATES EFFORTS TO ELIMINATE LYMPHATIC FILARIASIS IN CENTRAL AFRICA

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The Global Programme to Eliminate Lymphatic Filariasis aims to eliminate the infection in all 73 endemic countries by 2020. Most countries outside of Africa have completed mapping and mass drug administration (MDA) is underway or even completed. In contrast, many countries in Africa have not yet started MDA and mapping data are often inaccurate or incomplete. Accurate, fine-grained mapping is especially important in Central Africa, because ivermectin used in MDA sometimes causes serious adverse events in persons with heavy *Loa loa* infections. Although the Democratic Republic of Congo (DRC) is believed to have the highest LF burden in Central Africa, implementation of MDA for LF has been delayed because of inadequate mapping of LF and because of coendemic loiasis. The aim of this study was to assess the prevalence of LF in Mambasa territory in northeastern DRC prior to implementation of MDA. Microfilaria testing was performed with 60 µL night blood smears, and *Wuchereria bancrofti* antigenemia was detected by the ICT card test. 1,226 subjects (>5 ys) were tested in 14 villages. Slides containing large sheathed microfilariae (Mf) were sent to WUSM for speciation of Mf by microscopy and by qPCR. Prevalence rates for *Mansonella perstans* (with thin, unsheathed Mf) ranged from 44 to 98%. Prevalence rates for *W. bancrofti* antigenemia ranged from 0 to 10% and exceeded 1% in 13 villages. Prevalence rates for large, sheathed Mf (either *L. loa* or *W. bancrofti*) ranged from 10-26%. Some samples had > 20,000 large Mf/ml, and 14% of those with large Mf had > 2,000 large Mf/ml. It is very difficult to verify the absence of *W. bancrofti* Mf by microscopy in thick smears that also contain high numbers of *L. loa* Mf. qPCR testing showed that 183 of the 184 blood samples with large Mf were positive for *L. loa*; 18 of these slides contained *W. bancrofti.* One slide contained Mf of *W. bancrofti* and *M. perstans* without *L. loa*. Since *L. loa* Mf have diurnal periodicity, it is likely that loiasis prevalence rates and infection intensities in the study area are much higher than those detected in night blood in our study. Since *W. bancrofti* antigenemia and Mf rates exceed 1%, Mambasa territory qualifies for MDA, although it may not be safe to use ivermectin in this area. This study illustrates difficulties associated with mapping and implementing MDA for LF in Central Africa.

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HEMOPARASITES IN CAPTIVE NON-HUMAN PRIMATES: RISKS FOR PUBLIC AND ANIMAL HEALTH

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Poaching and trade of wildlife for local and international exotic pet markets result in frequent close contact between human and non-human primates (NHP). This forced sympatry, coupled with the phylogenetic proximity of humans and NHPs, provides multiple new opportunities for disease emergence. Limited information is available about the presence of bloodborne parasites affecting NHPs in Latin America. Isolated reports have been published but there are no systematic, comprehensive and large scale studies. We conducted a prospective study to estimate the prevalence of blood-borne parasites in captive NHPs that could pose a potential risk for humans. NHPs were sampled in all major cities in the Amazon Basin (Yurimaguas, Iquitos, Pucallpa and Puerto Maldonado) and at two cities with major trade (Lima and Cuzco). All species found were studied, aiming at reaching at least 30 specimens per the three major genera, woolly (*Lagothrix* spp.), capuchin (*Cebus* spp.), and squirrel (*Saimiri* spp.) monkeys, most commonly found in captivity in Peru. Peripheral blood samples (1-3ml) from the femoral vein were collected from chemically restrained primates following verbal consent from their owners. Knott's concentration method and blood smears were performed. Slides were assessed by microscopy at NAMRU-6 by trained staff under the supervision of senior microscopists. Currently, 60 monkeys from zoos and wildlife rescue centers, 41 from wetmarkets and 34 household pet monkeys have been sampled. Forty-nine squirrel, 19 woolly, 33 capuchin, and 34 monkeys belonging to other species have been included. The frequency of microfilaremia was 55% for squirrel monkeys and between 0-33% in the other species. Mixed infections, including *Dipetalonema* spp. and *Mansonella* spp., were found in ten squirrel monkeys. Furthermore, trypanosomiasis was detected in *Saimiri sciureus* (4/25), *Lagothrix lagotricha* (1/9) and *Cebus apella* (1/7). Also, a *Plasmodium* spp. parasite has been found in one squirrel monkey. Wild-caught squirrel monkeys, confiscated from the pet trade, were positive for the three types of hemoparasites reported here, highlighting the risk of introducing these animals to urban settings through the pet trade. Our findings suggest that bloodborne parasites are highly prevalent among Peruvian NHPs and may pose a serious risk to public and animal health that has been largely understudied.

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LYMPHATIC FILARIASIS TRANSMISSION IN 3 ECO-CLIMATIC AREAS AND THE INNER DELTA OF NIGER RIVER IN MALI

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Lymphatic filariasis (LF) is a public health problem because of its serious sequel including elephantiasis and hydrocele. Given the endemicity of LF in all regions of Mali, the potential variations of the endemicity level according to the eco-climatic areas and the need of a better use of scarce resources, a parasitological and entomological study has been conducted in 3 eco-climatic areas and the inner delta of Niger River in Mali. A cross-sectional study was undertaken for the parasitological aspect and consisted to make 3 slides of night thick smear of 20 *ul* of blood each. Volunteers' brief medical examination for LF disabilities (hydrocele and elephantiasis) was done at the beginning. For entomological assessment of LF transmission was done through mosquito collection using Human Landing catch (HLC) in August, October and December 2010 in two villages of each of the three eco-climatic areas over the five existing in Mali: southern Sudan, northern Sudan, and Sahelian areas and one village in the Niger river inner delta (Rice growing area). The collected vectors infection rate was determined using a PCR on pools of 20 mosquitoes. Collected mosquitoes (*Anopheles gambiae s.l* and *An. funestus*) were stored in pools of 20 before the PCR essay aimed at detecting Wb DNA and infection rate estimation using the Poolscreen 2 software. Of the 1,017 subjects tested in the 4 ecoclimatic areas, the wb mf prevalence was 16% in the southern Sudan area, (15.4%) in the northern sudan area and 0% in the Sahelian and Niger inner delta areas. The prevalence of Hydrocele was 5.81 % in north Sudan area, 1.35 % in south Sudan area and 0% in the Sahelian and the Niger inner delta areas. The prevalence of elephantiasis was 3.5% in southern Sudan area, 0.3% in the sahelian area, 0.4 in the northern sudan area and 0% in the Niger inner delta areas. Over the 888 pools of mosquitoes processed by PCR, the average infection rates with 95% confidence interval were 2% (1.2-4.9), 0.4% (0.2-0.9), 0.2% (0.1-0.4), 0.2% (0.1-0.4) respectively in the southern sudan, the northen sudan, the Niger inner delta and the sahelian areas. The north and south Sudan areas are the most endemic for LF and should attract the attention of the decision makers in the context of LF elimination in term of resources allowance.

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URBAN RISK MAPPING OF LYMPHATIC FILARIASIS IN DAR ES SALAAM, TANZANIA

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Little is known about the magnitude and distribution of lymphatic filariasis (LF) in coastal urban areas of East Africa, where the disease is caused by *Wuchereria bancrofti* and primarily transmitted by *Culex quinquefasciatus*. This study aimed to map LF prevalence across Dar es Salaam, Tanzania, and determine the local knowledge of disease, potential risk factors and activities of the National LF Elimination Programme in preparation for a large scale-up of mass drug-administration (MDA). A human infection survey and household semi-structured questionnaire were carried out in 10 urban and peri-urban locations (wards) across the three municipalities of the city. LF prevalence of Circulating Filarial Antigen (CFA) was determined using Immunochromatographic test (ICT), and microfilaria (Mf) counts were examined in ICT positive individuals. Evidence of clinical disease was also recorded. In total, 1591 individuals from 259 households were tested for LF infection and 8.5% (n=141) were found to be ICT positive (all Mf=0). Prevalence varied between locations ranging from 2.2% in Buguruni (sub-ward Madenge=0%) to 14.0% in Bunju (sub-ward Bunju A=18.1%). More males (n=85) than females (n=56) tested ICT positive, and individuals < 35 years (61.7%) were the most affected age-group. In total, only 3 cases of lymphodema and 5 hydroceles were recorded. The household questionnaire indicated that 63.2% of those interviewed had heard of LF, but 58.9% did not know the main symptoms, and 61.3% did not know the cause. Approximately half (51.2%) knew about LF Elimination Programme, and one third (35.2%) knew about MDA, with health workers and community members being the most common source of information (>50%). The results and risk maps developed from this study will help the Neglected Tropical Disease (NTD) Programme to implement MDA with special emphasis on advocacy and sensitization of the communities on the disease and the LF Programme itself.

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FROM CONTROL TO ELIMINATION OF AFRICAN ONCHOCERCIASIS: SHOULD THE FREQUENCY OF IVERMECTIN MASS TREATMENT BE INCREASED?

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The African Programme for Onchocerciasis Control (APOC) has implemented annual mass treatment in areas of African where onchocerciasis is mesoendemic or hyperendemic, aiming to reduce transmission and prevent morbidity. In view of the recent shift in focus from morbidity control to elimination of African onchocerciasis where possible, it has been suggested that APOC should increase the frequency of mass treatment from annual to semiannual, to speed up elimination. To assess under which circumstances this could be worthwhile, we simulated the outcome of annual and semiannual mass treatment in terms of the number of treatment rounds, calendar time and amount of drugs required for elimination. Simulations were done for different settings pertaining to pre-control infection levels and history of mass treatment (number of rounds and population coverage). Simulations were performed in ONCHOSIM, an established microsimulation model for onchocerciasis transmission and control. We updated assumptions about ivermectin efficacy in ONCHOSIM, based on a systematic review of literature regarding the effects of repeated treatments with ivermectin on onchocerciasis, and in particular, adult *Onchocerca volvulus* worms. ONCHOSIM predicted that semiannual treatment (vs. annual) would reduce time required until elimination, especially in foci where mass treatment was started only recently (time reduction of up to 50%). This benefit was lower for foci with high pre-control prevalence of infection or a history of high mass treatment coverage, and foci where coverage of semiannual mass treatment turns out lower than expected. Furthermore, the semiannual treatment strategy required up to 5 more treatment rounds until elimination than the annual treatment strategy. In conclusion, increasing frequency of ivermectin mass treatment in APOC areas may have considerable benefits, depending on several factors. These benefits will have to be weighed against investments and possible logistical barriers for increasing the frequency of mass treatment.

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HOUSEHOLD-LEVEL RISK MAPPING OF LYMPHATIC FILARIASIS IN URBAN AND PERI-URBAN AREAS OF MOMBASA, KENYA

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Lymphatic filariasis (LF) is endemic in the coastal areas of Kenya where it is caused by Wuchereria bancrofti, and transmitted by *Anopheles* and Culex mosquitoes. The National LF Elimination Programme is implementing mass drug administration (MDA) using albendazole and diethylcarbamazine citrate (DEC) to interrupt the transmission, however, it is not known if this needs be extended to the city of Mombasa because of lack of data on the disease. The aim of this study was to map the prevalence of LF across urban and peri-urban areas of Mombasa, and determine the local knowledge of disease and the LF Programme. A human infection survey and household semi-structured questionnaire were carried out in 5 areas of the city. LF prevalence was determined using Immunochromatographic test (ICT), and microfilaria (Mf) counts in ICT positive individuals. Evidence of clinical disease was also recorded. All data were mapped to household level using geographical coordinates collected during the survey. In total, 510 individuals from 129 households were tested and 2.4% (n=16) were found to be ICT positive. MF counts ranged from 0 to 8. More females (n=10) than males (n=6) tested ICT positive, and ages ranged from 8 to 68. Households with ICT positives tended to be clustered within each area. In total, 5 cases of lymphodema and 6 hydroceles were reported. The household questionnaire indicated that 83.6% of those interviewed had heard of LF, but 57% did not know the main symptoms, and 47.7% did not know the cause. Approximately one third knew about the LF Elimination Programme (30.5%), and about MDA (33.9%), with TV being the most cited source of information (>20%). These results indicate a low sporadic level of LF endemicity in Mombasa and a challenge for the LF Elimination Programme if they were to implement MDA with high coverage.

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SECONDARY MAPPING OF LYMPHATIC FILARIASIS IN HAITI-DEFINITION OF TRANSMISSION FOCI IN LOW-PREVALENCE SETTINGS

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To eliminate Lymphatic filariasis (LF) as a public health problem, the World Health Organization (WHO) recommends that any area with infection prevalence greater than or equal to 1% (denoted by presence of microfilaremia or antigenemia) should receive mass drug administration (MDA) of antifilarial drugs for at least five consecutive rounds. Areas of low-antigen prevalence (< 1%) are thought to pose little risk for continued transmission of LF. Five low-antigen prevalence communes in Haiti, characterized as part of a national survey, were further assessed for transmission in this study. An initial evaluation of schoolchildren was performed in each commune to identify antigen-positive children who served as index cases for subsequent community surveys conducted among households neighboring the index cases. Global Positioning System (GPS) coordinates and immunochromatographic tests for filarial antigenemia were collected on approximately 1,600 persons of all ages in the five communes. The relationship between antigen-positive cases in the community and index cases was evaluated using multivariate regression techniques and analyses of spatial clustering. Community surveys demonstrated higher antigen prevalence in three of the five communes than was observed in the original mapping survey; autochthonous cases were found in the same three communes. Regression techniques identified a significantly increased likelihood of being antigen-positive when living within 20 meters of index cases when controlling for age, gender, and commune. Spatial clustering of antigen-positive cases was observed in some, but not all communes. Our results suggest that localized transmission was present even in low-prevalence settings and suggest that better surveillance methods may be needed to detect microfoci of LF transmission.

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LYMPHATIC FILARIASIS ELIMINATION IN NIGERIA: RESULTS OF THE TREATMENT ASSESSMENT SURVEY (TAS)

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The current strategy for interrupting transmission of lymphatic filariasis (LF) in Africa is annual mass drug administration (MDA) with ivermectin and albendazole for 6 or more years. In Plateau and Nassarawa states in north-central Nigeria, MDA was started in 2000 and reached full

coverage in all 30 local government areas (districts) by 2003. In 2008, after 6 rounds of MDA, a large (>30,000) two-state survey assessing district level antigenemia showed that transmission had been interrupted in 10 of the 30 districts, but MDA needed to continue in the remaining districts. By early 2011, long lasting bed nets (LLIN) had been distributed at household level in the two states by the malaria program. In 2012, new WHO guidelines for stopping LF MDA were issued. They called for cost savings by conducting a 'TAS' in grouped 'evaluation units' where districts of similar epidemiological characteristics are joined. We grouped the remaining 20 MDA districts into four EUs (two in each state) based on the 2009 survey results. Districts with an overall 2008 antigenemia of ≤2% were joined (called Group 1) and district >2% antigenemia were joined (Group 2). Our assumption was that Group 1 EUs were most likely to 'pass the TAS' and move on to stop MDA and enter into post MDA surveillance. A cluster survey was conducted of primary-school children (6-7 years of age) based on an algorithm provided in the TAS manual that called for a minimum sample size of 1692 per EU with a critical cut off point of 20 positives per EU resulting in failure for that EU. A total of 7,131 children were tested in 173 schools: 43 schools per EU. Group 1 in both Plateau and Nassarawa passed with 8 and 3 ICT positives respectively, and are able to stop MDA. Group 2 also passed in both states with only 10 and 3 positives. Challenges faced that will be discussed included civil unrest and government labor strikes.

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INTERRUPTION OF ONCHOCERCIASIS TRANSMISSION IN THE ABU HAMED FOCUS, NORTHERN SUDAN

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The isolated Abu Hamed focus in Northern Sudan represents the northernmost endemic area of onchocerciasis in the world with 118,034 individuals at risk. Communities in the focus have undergone annual community-based treatment with Ivermectin (CDTI) with variable coverage rates since 1998. The CDTI control program has been upgraded to biannual treatments in 2006 with baseline entomological, parasitological and serological data collected in 2007. Follow up assessments of biannual treatments and CDTI were performed in 2011. *Onchocerca volvulus* O-150 Pool screen analysis showed no parasite DNA in 16,489 *Simulium damnosum* flies collected from Dec 2010 to Nov 2011 (95% confidence interval upper limit (UL) = 0.042 per 2000 flies) compared to 0.168 (0.0.099-0.376 95% CI) in 2007. Screening for skin snips and onchodermatitis in 536 individuals in 8 villages in and around the focus revealed no microfilariae or skin disease (95% CI UL = 0.0075%) in contrast to mf rate of 0.45% in 2007. Similarly, sera collected from 6,756 school children ≤10 years old showed no evidence of *O. volvulus* Ov16 IgG4 antibodies (95% CI UL = 0.0007%) compared to zero (0-1.36% 95% CI) prevalence of the antibodies in the same age group in 2007. Our 2011 assessment of the Abu Hamed focus has met the criteria for interrupted transmission of the disease in the area. CDTI activities were halted in 2012 and post-treatment surveillance (PTS) period of three year was initiated. Challenges to the elimination program and plans for PTS will be discussed.

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PREVENTION OF TYPE 1 DIABETES BY *LITOMOSOIDES SIGMODONTIS* INVOLVES MULTIPLE MECHANISMS

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Prevention of Type 1 diabetes (T1D) is a major focus of research in our laboratory. We use the rodent filarial pathogen *Litomosoides sigmodontis* (LS) to understand protective mechanisms against T1D. We have shown that LS infection prevents T1D and transfer of splenocytes from infected NOD mice into NOD/scid mice fails to induce T1D. We hypothesized that this results from altered T-cell populations. Flow cytometric analysis of uninfected and infected spleens showed no difference in total frequencies of naïve or memory CD4+ T-cells (41/20% vs. 43/20%, p=0.9/0.6) or autoantigen BDC2.5-specific naïve and memory CD4+ T-cells (0.01/0.01% vs. 0.007/0.01%, p=0.3/0.9), suggesting that LS may alter function rather than number of autoimmune cells. Helminth secreted products are known immunomodulators. We recently showed that LS protection against T1D depends on TGFβ. Therefore, we evaluated whether LS excretory/secretory products (ESP) signal through the mammalian TGFβ receptor. *In vitro* assays using a TGFβ responsive cell line show that ESP from microfilariae, L3s, male adult, and female adult worms ligate the TGFβ receptor. Additionally, four weekly injections of NOD mice with male or female ESP delays onset of T1D. The delay is greater in female than male ESP-treated mice (4 v. 2 weeks). Studies are ongoing to assess the functionality of autoimmune CD4+ T-cells during infection and to further evaluate the role of ESP in helminth-mediated protection against autoimmunity.

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VACCINATION AGAINST *BRUGIA MALAYI* CYSTEINE PROTEASE INHIBITOR(S) (BM-CPI) ALTERS THE *B. MALAYI* MIGRATORY PATTERN IN MONGOLIAN GERBILS

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Cysteine protease inhibitors are reversible, tightly binding inhibitors of cysteine proteases and are secreted by the filarial worms and have been shown to exhibit immunomodulatory properties and function in protective immunity. In human filarial parasite, *Brugia malayi*, 2 cysteine protease inhibitor genes, Bm-CPI-1 and Bm-CPI-2 have been described and Bm-CPI-2 has be shown to modulate the host immune response by inhibiting the multiple cysteine protease activities found in the endosomes/lysosomes of human B lymphocyte lines. In *Onchocerca volvulus* and *Litomosoides sigmodontis* animal models they were shown to elicit protection against infection with L3 larvae. Interestingly, excretory-secretory proteins (ES) of filarial nematodes have been known to also play an important role in immunomodulation and immune protection. For example, in the *B. pahangi* - Mongolian gerbil model system, immunization with ES resulted in a modified pattern of migration of L3s in comparison to the controls. In the ES immunized gerbils, more L3s were isolated at the site of infection compared to adjuvant control, suggesting that anti-ES immune responses limit larval migration. as reported previously. As both Bm-CPI-1 and Bm-CPI-2 are present in ES *of B. malayi*, we hypothesized that they could also play a role in protective immunity to *B. malayi* infection in gerbils, as judged by worm development and distribution in vaccinated gerbils. *E. coli* expressed recombinant Bm-CPI-1 and Bm-CPI-2 were used to immunize gerbils using alum as the adjuvant. Gerbils were challenged with L3s and parasites were recovered on day 42 post-infection. There was no significant reduction in worms in Bm-CPI-1 and Bm-CPI-2 immunized gerbils in comparison to alum only controls. However, both Bm-CPI-1 and Bm-CPI-2 vaccinated groups had more worms in the heart and lungs

than control gerbils and fewer worms were found in the lymphatics of the Bm-CPI-1 and Bm-CPI-2 vaccinated groups in comparison to controls. Especially with Bm-CPI-1 vaccination, these changes in distribution of worms were higher and statistically significant. Our results suggest that immunity induced by CPI-1 and-2 may have an effect on migration of worms away from the lymphatics as more worms are observed in heart & lungs of Bm-CPI-1 and-2 immunized gerbils. This phenomenon in relation to the immunomodulatory function of filarial cysteine protease inhibitors is further discussed.

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IMMUNOINFORMATICS APPROACH TO VACCINE DESIGN AGAIN LYMPHATIC FILARIASIS

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As the 2020 deadline for the eradication of lymphatic filariasis approaches, reliable prophylactic measures to fight against recrudescence and/or drug resistance may be in critical need. Currently, mass drug administration (MDA) rounds have been shown to be effective at reducing parasite burden and overall prevalence within participating endemic countries. Within such countries, MDA stopping points are being considered based on careful algorithms such as those used during transmission assessment surveys (TAS). However, TAS endpoint predictions have not been verified by any prior field validations. Therefore, recrudescence becomes an issue of concern. Therefore, the development of a vaccine could prove to be a more reliable means of prophylaxis. Furthermore, existing gene to vaccine immunoinformatics tools could be used to expedite the selection of highly immunogenic T-cell epitopes for the development of a DNA vaccine. Thus, a large-scale immunoinformatics screening approach was used for *Brugia malayi*, one of the three causative agents for LF, combining GenBank database of 11,465 proteins with EpiVax immunoinformatics software. Of these, 1,774 proteins demonstrated putative immunogenicity. Of these, 456 proteins were predicted to have extraordinary immunogenicity properties. Current studies also indicate homology of the *Brugia malayi* putative immunogenic proteins with *Wuchereria bancrofti* proteins and *Loa loa* proteins.

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GLYCAEMIC PROFILE ON HIV+ PATIENTS ON HAART IN KIGALI UNIVERSITY TEACHING HOSPITAL: A REVIEW OF 117 CASES

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HAART has been reported to be associated with new-onset diabetes mellitus .However the data available in Rwanda is scarce. This study aimed to determine the prevalence of diabetes mellitus in HIV-infected patients receiving ART. In this prospective study, HIV-infected patients on HAART who attained the clinic of Kigali University Teaching Hospital from February to August 2011 were studied. Fasting plasma glucose was performed in each patient. There were 117 patients with a mean age of 43.5 (range, 15 to 65) years and male were 46.2%. The most common risk factor was an habitual physical inactivity, retrieved in 42 patients, followed by an overweight status (BMI≥25 kg/m^2) for 36 patients (30.8%). The waist-to-hip ratio was abnormal in 16.7% of male patients and 70.3% of female patients of this study. 29 patients (24.8%) presented a lipodystrophy syndrome. Changes of glucose homeostasis have been observed in 48 patients (41%), 18 of them having diabetes mellitus (15.4%). No factor (either traditional risk factors or HAART regimen characteristics) appeared to have influenced the occurrence of impaired fasting glucose among the patients of this study. Glucose impairments are fairly high among HIV-infected patients receiving HAART, especially in those presenting a lipodistrophy syndrome. Therefore, those patients should be submitted to a regular Fasting Plasma Glucoses. However, a large-scale study is undoubtedly required to confirm our results.

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FACTORS INFLUENCING ADHERENCE TO ANTIRETROVIRAL THERAPY IN ADOLESCENTS AT BOTSWANA-BAYLOR CHILDREN'S CLINICAL CENTRE OF EXCELLENCE - A QUALITATIVE STUDY

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The aim of the study was to determine the factors that influence adherence to ART among adolescents who contracted HIV through vertical transmission. Qualitative research using descriptive phenomenology was conducted at Botswana-Baylor Children's Clinical Centre of Excellence. The meta-theoretical assumptions were that the experience of adolescents who are HIV positive and taking ART would put them in a position to give insight into what makes one to adhere or not to adhere to treatment and that the the critical truth can be found through in-depth individual semi-structured interviews about the factors that influence adherence to ART. Data was collected using in-depth individual semi-structured interviews. Eight (8) adolescents between 14 and 19 years who had been on ART for minimum of 4 years were interviewed. Thematic analysis of data was done and five (5) themes emerged from the participants' description of the experience of taking ART taking ART over a long period of time. The themes that emerged indicted the factors that influence adherence to ART, and they included knowledge and positive beliefs about ART, need for support, ART difficult treatment regimen, having a regular doctor and psychosocial emotional needs. The findings suggested that the adolescents who contracted HIV through vertical transmission require support while continuing on a simplified long-term ART regimen after an assessment of their psychological well beings and periodic checks.

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PREVALENCE OF MALARIA AMONG HIV SEROPOSITIVE CLINIC ATTENDANTS IN FIVE HOSPITALS IN GHANA

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Malaria is associated with an increase in viral load and fall in CD4-cell count. Conversely, HIV infection also disrupts the acquired immune response to malaria and the efficacy of antimalarial drugs. This study was carried out in five Ghanaian hospitals to estimate the prevalence of malaria among HIV patients and also to assess possible factors that influence the epidemiology of malaria and HIV co-infection. The descriptive cross sectional study reviewed and collected data on malaria, using Case Record Forms from HIV patients' folders in five hospitals in the Brong Ahafo and Ashanti Regions of Ghana. Ethical approvals were obtained from three recognized Ethical Review Committees. The total 933 patients were made of 272 (29.2%) males and 661 (70.8%) females. Majority were between 21- 41 (63.6%) years old. In the 933 folders reviewed, 339 (95.5%) of 355 were clinically suspected and referred to the laboratory for confirmation of malaria diagnosis. Only 4.4% (95% CI: 2.2, 6.6) patients tested were confirmed cases of malaria. Fever, was not significantly associated with confirmed malaria (OR=3.11, 95% CI: [0.63, 15.37], P=0.142), however, fever was presumptively used to diagnose

malaria (OR=4.11, 95% CI: [2.83, 5.96], P<0.001). Huge missing data were recorded due to the poor keeping of records at all the sites. In conclusion, a low prevalence of confirmed malaria, 4.4% (95% CI: 2.2, 6.6), was recorded among HIV patients from Ghana. The prevalence could also be attributed to the high cases of malaria diagnosed presumptively (37.0%), (OR=4.11, 95% CI: [2.83, 5.96], P<0.001). Evidence based diagnoses and treatment of malaria should be improved. Demographic characteristics, CD4 count levels and ART status of patients were not significantly associated with malaria. This may be due to the poor records keeping at all sites.

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EXTENT OF INADEQUATE RECORDS KEEPING AND THE EFFECT ON PATIENT CARE FROM FIVE HIV MANAGEMENT HOSPITALS IN GHANA

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Medical records serve many functions but their primary purpose is to support patient care and to improve in control programmes. The importance of completeness of patients' records cannot be overemphasized, especially for the purpose of auditing, research, and medico-legal reasons. However gross inadequacies are often noted. We carried out this review to observe the quality of records keeping in five HIV management clinics. The study reviewed 933 patients' folders randomly selected systematically from five hospitals in two regions of Ghana. Quality assessment was made on the extent of missing and incomplete records in the folders of patients. Case Record Form was developed to collect data on HIV and malaria from the management clinics. Ethical approvals were given by the Ethical Review Committees of Ghana Health Service, London School of Hygiene and Kintampo Health Research Centre and permissions from the hospitals. Data analyses were done using Stata 11 software. The study revealed extensive amount of missing records in patients' folders across the sites. The extent of missing data ranges from 3.5% on patients' initial CD4 counts to 90.5% of TB screening results and 100% on malaria preventive practices. Incomplete records were also collected on variables such age, weight, fever and treatments given. The quality of record keeping in the HIV management clinics was inadequate. This is comparable to what was observed by Peltzer et al, 2010. It is important not only for patients' care, but also to improve in the existing control measures. Though the case is argued for establishing evidence-based standards for record keeping, where such standards are present, we recommend regular audits and disciplinary measures to make sure health workers adhere to existing protocols for proper documentation. Also, adequate staffing should be provided to handle data collection at the various HIV management clinics in addition to the clinical staff.

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GENETIC ANALYSIS OF HIV-1 SUBTYPES IN RWANDA

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Human immunodeficiency virus type 1 (HIV-1) infection cases in Rwanda among adults are estimated to be 200,000, and among these more than 50% are on highly active antiretroviral therapy (HAART). The recent emergence of drug resistant HIV strains has created an urgency to evaluate the circulating HIV genotypes. As a result, the National Reference Laboratory of Rwanda and its collaborating partners have initiated a HIV genotyping study to monitor drug resistance in the infected population. The objective of the study was to determine the HIV subtypes among submitted samples and for which sequences were generated. Quantification of viral load (VL) in plasma samples was performed using Roche COBAS® AmpliPrep/Cobas® and Taqman® RealTime HIV-1 assays. Viral RNA was extracted using Viroseq v2.0 technology (Abbott) and sequencing was accomplished with the ABI 3500xl (Applied Biosystems). A total of 134 randomly selected nucleotide sequences were analyzed in the protease (PR) and reverse transcriptase (RT) regions with the HIVseq program (Stanford University) for classification based on subtype. Sequences for both PR and RT were generated for all samples. Among the 134 sequences analyzed in the PR region, 59 (44%) were circulating recombinant forms (CRF01_AE), 56 (41.8%) were subtype A, 15 (11.2%) were subtype C, 3 (2.2%) were subtype D, and 1 (0.8%) was subtype K. For the RT region, 18 (13.4%) were CRF01_AE, 91 (67.9%) were subtype A, 21 (15.7%) were subtype C, 4 (3.0%) were subtype D, and 1 (0.8%) was subtype K. Concordant PR/RT subtypes A, AE, C, D, and K were found in 46 (34%), 11 (8.2%) 15 (11.2%), 3 (2.2%), and 1 (0.8%) respectively. Genetic tree of HIV1 in Rwanda. In conclusion, this is the first study to extensively evaluate the HIV-1 strains in Rwanda in the *pol* gene region and to report on HIV-1 subtype K. Our RT data support observations by others that about 70% of HIV-1 in Rwanda is subtype A. However, our PR data indicate that the prevalence of subtype A and CRF01_AE is comparable at 44.0% and 41.8% respectively. Overall, more than 80% of HIV-1 circulating in Rwanda are either subtype A or CRF01_AE in the pol region.

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BAFFLED BY FALLING OR STAGNATED CD4 COUNTS IN YOUR PATIENTS ON ANTIRETROVIRAL THERAPY (ART) DESPITE ADEQUATE HIV VIRAL SUPPRESSION? DO NOT IGNORE BRUCELLOSIS

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Brucellosis is the commonest zoonotic disease in the world being endemic or re-emerging in many areas including Africa. We noticed a significant decline or stagnation in CD4 counts in some of our clients who were adherent on their ART and had no evidence of active pulmonary tuberculosis (PTB).Then we got the landmark case of brucella meningo-encephalitis in AIDS that prompted this case study. She had been on ART for 24 months with an undetectable viral load (<400 copies/ml) but the CD4 counts fell from 565 to 310 over 8months and developed delirium plus seizures. Serology and cerebrospinal fluid examination identified neurobrucellosis and cyptococcal meningitis as well (despite her counts!). After anti- brucella treatment the counts bounced back to 610 and the neurological features cleared. This suggested that brucellosis was responsible for the phenomenon so we began seeking other cases. Of 1400 patients on ART, 21 had declining/stagnated counts despite good adherence. Fourteen had either brucellosis or urinary TB but detectable viral loads (>400 copies/ml) (excluded), 5 had undetectable viral loads and brucellosis (included in study),2 had undetectable virus with urinary tuberculosis (excluded). All the 5 included were female with duration on ART of 10 to 42 months. Two had spondyloarthritis,2 had neurobrucellosis and 1 had enteritis. Four had CD4 drops that ranged between 10 and 45 %. One had stagnated at 185 counts despite being on ART for more than 20 months. All got 12 weeks of Doxycycline plus Cotrimoxazole and 2 weeks of Streptomycin and their CD4 counts normalized. Our study shows that brucellosis in AIDS patients on ART, can cause CD4 counts to significantly fall /stagnate despite the HIV virus being adequately suppressed. Patients who have this situation should be screened for brucellosis. Bigger studies on the impact of brucellosis on HIV/AIDS and effectiveness of ART are needed.

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SURROGATES OF HIV DRUG RESISTANCE MUTATIONS IN PATIENTS FAILING FIRST LINE THERAPY IN JOS, NIGERIA: AN INVALUABLE TOOL IN RESOURCE CONSTRAINT COUNTRIES

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Nigeria with a population of 160 million people has HIV prevalence of 4.1% accounting for 10% of the world HIV burden. There has been a rapid scale up of antiretroviral therapy in Nigeria from <10,000 in 2002 to over 300,000 patients in 2010. The emergence of antiretroviral drug resistance is now a major challenge to successful treatment. Development of drug resistance in Nigeria like in other developing countries is facilitated by the initial erratic supply of HIV drugs, stigma, poverty, poor adherence, inadequate infrastructure and insufficient human resources. Drug resistance testing is largely unavailable in resource constraints countries. There is therefore the need to determine surrogates that are available in resource constraint countries. We hypothesized that the prevalence antiretroviral drug resistance mutations among patients failing first line antiretroviral drugs in our setting was high and that declining CD4 counts, decreasing weight, HIV viral load ≥4log copies/ml (at virological failure), duration on antiretroviral therapy >1 year, poor adherence and presence of opportunistic infections (at virological failure) were surrogates of antiretroviral drugs resistance mutations. It was a cross sectional study carried out the in Jos, one of the largest HIV treatment site in Nigeria. The study was carried out between July and October, 2010. One hundred consenting patients were randomly selected from 320 patients that had virological failure. Multiple Logistic regression analysis was used to determine factors independently associated with antiretroviral drug resistance.The overall prevalence of antiretroviral drug resistance was 82%. HIV viral load ≥4log copies/ml(at the time of virological failure) (P=0.01), duration on antiretroviral therapy >1 year (P=0.03) and presence of opportunistic infections (P=0.03) were found to be associated with drug resistance mutations. These may be used in resource poor settings as surrogates' of antiretroviral drugs resistance mutations.

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BARRIERS TO UTILIZATION OF PROVIDER-INITIATED HIV COUNSELING AND TESTING SERVICES AMONG TUBERCULOSIS PATIENTS: A CASE OF RHODES CHEST CLINIC NAIROBI, KENYA

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Tuberculosis (TB) continues to be one of the most important global public health threats particularly in Sub-Saharan Africa where majority of TB patients are also infected with HIV. HIV prevalence among TB patients in sub-Saharan Africa is 70 %. In 2007, WHO recommended that countries with high co-infection rates develop TB and HIV collaborative activities, including provider-initiated HIV testing and counseling (PITC) of TB patients in TB clinical settings. The objective of the study was to determine the barriers to utilization of provider initiated HIV counseling and testing services among TB patients. A cross sectional survey of TB suspects visiting Rhodes chest clinic, Nairobi was conducted. Consenting patients who visited the clinic during October to December 2010 were the study subjects. Data was collected using a standard questionnaire. A chi square test was used to interpret results for each possible barrier in terms of utilized versus declined to utilize HIV counseling and testing services. Written informed consent was obtained from all participants. Eighty three percent of TB patients tested for HIV infection. The main reasons for not being tested were that they don't trust confidentiality (17.9%), fear of positive test results (11.9%), fear of discrimination (10.4%) and self perception of low risk (7.5%) (χ2=29.473, 9 df, p=0.030). Factors that were significantly associated with utilization of PITC services were age (χ2=11.319,2df,p=0.003), gender (χ2=5.919,1df,p=0.015), level of education (χ2=116.045,2df,p=0.0001), HIV stigma (χ2=36.947,3df,p=0.0001), awareness of HIV-TB link (χ2=22.767,2df,p=0.0001) and discussion of HIV/ TB link by nurse (χ2=59.232,2df,p=0.0001). In conclusion, utilization of PITC services by TB patients was high at the Chest clinic. The Kenya National AIDS Control Council, National Leprosy and Tuberculosis Program (NLTP) and TB/HIV Partners should scale up community awareness about HIV-TB co infection and train all providers on collaborative HIV-TB services. Advocacy for HIV screening for all TB patients should also be increased.

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MONITORING OF SEXUALLY TRANSMITTED INFECTIONS IN SENEGAL: A NATIONAL SURVEY CARRIED OUT IN 2006 AND IN 2010 AMONG RESPECTIVELY 596 AND 570 FEMALE SEX WORKER IN SOME STIS CENTRES OF SENEGAL

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This work aimed to determine the prevalence of sexually transmitted infections (STI) in the group of the Female Sex Workers (FSW) in Senegal. We have done a behaviour survey combined to a biological investigation. The vaginal specimen collection was done by the FSW herself and blood was collected at the elbow. The vaginal secretion was used for the diagnosis of vaginal candidasis, *Trichomonas vaginalis* vaginitis and bacterial vaginosis; blood was analyzed for diagnosis of syphilis and the HIV infection. On a total of 1166 women, 596 were tested in 2006 and 570 in 2010. The age varied from 15 to 62 years with an average of 35,09 years in 2006 and 36,59 years in 2010. All were Female Sex Workers registered with in a national medical centre or working as a clandestine. *T. vaginalis* was found in 11, 2% of the cases in 2006 and 14, 9% of the cases in 2010 with a non significant difference whereas the prevalence of the vaginal candidasis was 7, 6% in 2006 and 11, 1% in 2010. The bacterial vaginosis affected 38,9% in 2006 and this STI has affected more than the half of the FSW in 2010 (52,3%). Bacterial vaginosis was almost always associated with a local inflammatory reaction. The rate of Syphilis went from 12,9% to 4,0% in 2010. The HIVprevalence was 19,9% among the FSW in 2006 and 11,9% in 2010 whereas the pregnant women tested in 2006 gave only a rate of 0,8% (0,6 to 4,3% according to area), thus confirming the HIV concentrated epidemic in Sénégal. Among the FSW, HIV co-infection with bacterial vaginosis were noted in 57% and 61,8% respectively in 2006 and 2010. This work shows the stability of HIV infection in Senegal but also it suggests some future orientations in care, support, prevention and research on STIs. It shows that the microbiological part of the national survey and monitoring of STIs must be conducted in a sentinel manner.

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IMPACT OF DEWORMING ON HIV AND VACCINE SPECIFIC ANTIBODY RESPONSES AMONG *ASCARIS* AND HIV CO-INFECTED ADULTS

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In Africa, individuals are frequently co-infected with helminths and HIV. Both infections impact the host immune response through immune suppression and dysregulation. As a result, these infections may also lead to diminished vaccine efficacy in populations where co-infection is prevalent. We determined the impact of deworming on humoral responses to HIV infection and previously administered vaccinations within a nested cohort study. HIV and *Ascaris* co-infected individuals were randomized to 400mg albendazole for 3 days (n=17) or placebo (n=19). Antibody responses to HIV antigens were measured using protein microarray before and 3 months post-treatment. Of the 132 HIV antigens examined, 21 were sero-reactive. Among sero-reactive antigens, there was no difference in average change in signal intensity over 3 months between the treatment groups (all p>0.20). In addition, vaccine antibody responses were measured using commercially available ELISAs to tetanus and measles among dewormed (n=16) and placebo treated (n=19) individuals. All were sero-positive (>0.01 IU/mL) for tetanus IgG at baseline and 3 months. The average change in tetanus IgG response over 3 months was -0.076 IU/mL in the dewormed group and +0.043 IU/mL in the placebo group (p=0.46). At baseline, 69% (n=11/16) of the dewormed group and 79% (n=15/19) of the placebo group were sero-positive for measles IgG. All of the dewormed individuals (n=11/11) compared to 80% of placebo treated individuals (n=12/15) remained sero-positive after 3 months (p=0.25). Additionally, 20% of dewormed (n=1/5) compared to 0% of placebo treated individuals (n=0/4), who were sero-negative at baseline sero-converted to positive after 3 months (p>0.3). Our study lacked sufficient power to detect an immediate effect of deworming on HIV or vaccine responses; however, short or long term impacts may still be important. If treating existing helminth infections modifies host responses to other antigens, greater efforts to empirically deworm in endemic areas may favorably impact responses to immunizations in these regions.

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HOST IRON STATUS AND IRON SUPPLEMENTATION IMPACT ERYTHROCYTIC STAGE OF *PLASMODIUM FALCIPARUM*

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Iron deficiency is prevalent in children and pregnant women in developing countries, and the World Health Organization recommends routine iron supplementation of children and adults. However, recent evidence suggests that iron deficiency may protect against malaria and that iron supplementation may increase susceptibility to malaria, complicating recommendations for universal supplementation in malaria-endemic regions. The biological mechanisms for both the malaria-protective effect of iron deficiency and the increased risk of malaria associated with iron supplementation remain unclear. To investigate the interactions between host iron status, iron supplementation, and the erythrocytic stage of *Plasmodium falciparum*, we used the *in vitro* model of the erythrocyte stage of *P. falciparum* infection to investigate the effect upon *P. falciparum* erythrocyte invasion and intraerythrocytic growth of (i) host iron status and (ii) iron supplementation of the host. This approach has allowed us to systematically study the *in vitro* growth, development and invasion efficiency of *P. falciparum* in red blood cells from iron-replete healthy and individuals with iron deficiency anemia, before and after iron supplementation. We observed decreased growth of *P. falciparum* in microcytic RBCs from individuals with iron deficiency anemia as compared to RBCs from iron-replete individuals. We found that *P. falciparum* matured normally through its 48 hour life cycle, but had decreased invasion efficiency into microcytic RBCs from individuals with iron deficiency anemia. When both iron-replete and iron-deficient individuals were given iron supplementation, we observed increased growth of *P. falciparum* in RBCs from individuals with iron deficiency anemia but not in iron replete individuals. It was observed that individuals with iron deficiency anemia receiving iron supplementation had elevated reticulocyte counts, and these reticulocytes had an increased parasite prevalence compared to mature RBCs. Finally, we show that *P. falciparum* is capable of invading and growing within RBCs of all ages, but that *P. falciparum* invasion efficiency and growth steadily decrease with increasing RBC age. These results suggest that host iron status and iron supplementation conspire to mediate host susceptibility to *P. falciparum* infection by altering the dynamics of the RBC population.

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SATA3 REGULATION OF MMP3 AND CEBPB IN MALARIA-INDUCED BRAIN DAMAGE

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Our previous results showed that STAT3 is activated by *Plasmodium berghei* ANKA infection *in vivo* and Heme *in vitro*. Heme up-regulates HO-1 and CXCL10 production through STAT3 pathway, and regulates CXCL10 at the transcriptional level *in vitro*. Mutual regulation exists between HO-1 and CXCL10. Since brain endothelial is one of the crucial components of blood brain barrier (BBB), which is severely disrupted and is a characteristic manifestation in cerebral malaria (CM), so we further determine how STAT3 signaling pathway is regulated in the pathogenesis of CM not only using experimental CM model and but *in vitro* human brain microvascular endothelial cells (HBVEC) culture. *1)* We analyzed the apoptotic effects of Heme on HBVEC using cell viability and TUNEL assays. *2)* We then analyzed Heme-induced JAK/STAT signaling pathway using real time RT^2 Profile PCR arrays. *3)* We next examined the effects of STAT3 on MMP3 by introduction of constitutive STAT3 (caSTAT3) and dominant STAT3 (dnSTAT3) into HBVEC. *4)* Lastly we measured the expression level of MMP3 in brain tissue in ECM model. Results of these studies demonstrate that *1)* Heme causes cell death in HBVEC by increasing apoptosis rate by 20% compared to control. JAK2 inhibition by AG490 protects HBVEC death induced by Heme. During this process, STAT3 is activated indicated by expression of phosphorylated STAT3 (pSTAT3) along with increased levels of MMP3, CXCL10 and HO-1. *2)* The results of real time RT^2 Profile PCR arrays are expressed as the fold changes in expression obtained by comparing HBVEC treated with Heme or with vehicle as control. The up-regulated genes (with fold-change greater than 2) include STAT-induced gene matrix metallopeptidase 3 (MMP3), apoptosis-related gene CCAAT/enhancer binding protein (C/EBPb), Fc fragment of IgG, high affinity Ia, receptor (FCGR1A), Jun B proto-oncogene (JUNB), nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 (NFκB), suppressor of cytokine signaling (SOCS3), SOCS4 and signal transducer and activator of transcription 4 (STAT4). The down regulated genes consist of coagulation factor II receptor (F2R) and 2′-5′-oligoadenylate synthetase 1 (OAS1). *3)* caSTAT3 up-regulates whereas dnSTAT3 down-regulates MMP3 in HBVEC treated by Heme. *4)* MMP3 and CEBP are up-regulated in brain tissues of C57 BL/6 mice infected with *P. berghei* ANKA. In conclusion, MMP3 and CEBPbeta which are the two down-regulators of STAT3 pathway might be highly related to malaria-induced damage.

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PRETERM DELIVERY IS ASSOCIATED WITH PREGNANCY MALARIA AMONG WOMEN LIVING IN OUELESSEBOUGOU, MALI

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Over many years of research it has been shown that adult residents of malaria endemic areas have developed immune responses that protect them from severe disease. Women become more susceptible to malaria during pregnancy, especially during the first pregnancy. To investigate the impact of pregnancy malaria on birth outcomes, pregnant women were recruited to a longitudinal study in Ouelessebougou Mali. The women were seen monthly and at any time they were sick by the clinical team. Gestational age was determined by ultrasound. Pregnancy malaria was defined as positive blood smear during pregnancy and placenta blood smear at delivery. The relationship between malaria infection and gestational age was assessed by logistic regression. After excluding twins and still-birth deliveries, the analytical population included 321 mother-infant pairs. Pregnancy malaria was more frequent among first time mothers with more than 50% of the women experiencing at least one malaria episode during pregnancy compared to 28% in multiparous mothers (OR = 3.1 (1.8-5.2), p<0.0001). Similarly, preterm deliveries were more common among malaria-infected primigravidas than multigravidas (34% and 9% respectively). Pregnancy malaria was associated with increased risk of preterm delivery after adjusting for the follow-up time in first time mothers (OR 3.27 (1.01-10.62), p=0.048). In summary, preliminary analysis of a cohort of women living in an area with seasonal malaria transmission suggests that pregnancy malaria is associated with risk for preterm delivery.

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IDENTIFICATION OF A NOVEL ERYTHROCYTE-BINDING LIGAND OF *PLASMODIUM VIVAX* MEROZOITE SURFACE PROTEIN 1 PARALOG, PVMSP1P

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Merozoite surface protein 1 of *Plasmodium vivax* (PvMSP1), a glycosylphosphatidylinositol-anchored protein (GPI-AP), is a malaria vaccine candidate for *P. vivax*. The paralog of PvMSP1, named *P. vivax* merozoite surface protein 1 paralog (PvMSP1P; PlasmoDB PVX_099975), was recently identified as a GPI-AP. Similar genetic structural characteristics between PvMSP1 and PvMSP1P (e.g., size of open reading frames, two epidermal growth factor-like domains, and GPI-anchor motif in the C-terminus) led us to study this protein. All of the PvMSP1P fragments including 83- (A, B, and C), 30-, 38-, 42-, 33-, and 19-kDa fragments predicted by the processed structure of PvMSP1 were expressed successfully as recombinant proteins. We studied the naturally acquired immune response against each fragment of recombinant PvMSP1P and the potential binding ability of each fragment to erythrocytes. The N-terminal (83A) and two C-terminal fragments (33 and 19) reacted strongly with antibody of sera from *P. vivax*-infected patients, producing 50-68% sensitivity and 95-96% specificity, respectively. An *in vitro* cytoadherence assay showed that PvMSP1P, especially the C-terminal 19-kDa region, could bind to erythrocytes. We also found that human sera from populations naturally exposed to *vivax* malaria and antisera obtained by immunization using the recombinant molecule PvMSP1P-19 inhibited *in vitro* binding of human erythrocytes to MSP1P-19. These results provide further evidence that the MSP1P is an essential parasite adhesion molecule in the *P. vivax* merozoite and is a vaccine candidate against *P. vivax*.

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EXPORTED PROTEIN-2 IS ASSOCIATED WITH MEMBRANE-BOUND VESICLES IN THE CYTOSOL OF *PLASMODIUM YOELII* 17X INFECTED RETICULOCYTES

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Malaria parasites replicate inside RBCs and export hundreds of proteins into the host cytosol in order to perform various functions including nutrient import and modification of the host cell cytoskeleton. A subset of these proteins is exported to the host cell surface where they mediate tissue-specific binding of infected RBCs to vascular endothelium. These surface-expressed parasite proteins represent potential vaccine targets as antibodies against them could block parasite sequestration, enhance phagocytosis and/or promote complement mediated lysis of infected RBCs. To identify parasite-encoded proteins exported to the host membrane, we performed a proteomic analysis of membrane proteins in *Plasmodium yoelii* 17X infected reticulocytes which identified eight *Plasmodium* proteins including the 31 kDa *P. yoelii* Exported protein-2 (PY05892). Full length Exported protein-2 (Exp-2) was cloned from cDNA and expressed as a recombinant protein fused to 6xHis-tag in *E. coli* BL21(DE3) Codon Plus cells. Recombinant Exp-2 was purified from the insoluble fraction by Nickel-NTA chromatography and polyclonal sera was raised against the protein in rabbits and mice. Antibodies against Exp-2 were able to detect the full-length native protein in the membrane fraction of *P. yoelii* 17X infected reticulocytes. Indirect immunofluorescence studies showed that Exp-2 was expressed in all blood stages, localized to the parasitophorous vacuolar membrane (PVM) and was also exported into host cytoplasm on membrane-bound vesicles budding from PVM. Budding of Exp-2 bound vesicles starts as early as ring stages and the number of vesicles increases during the trophozoite and schizont stages. Studies in *Plasmodium falciparum* identified Exp-2 as part of the PTEX translocon machinery present on the PVM where it is involved in export of parasite-derived proteins. However, in our *P. yoelii* rodent model, we show that Exp-2, in addition to PVM localization, is exported into the reticulocyte cytoplasm suggesting a potential role in protein export beyond the parasitophorous vacuolar membrane. Studies are ongoing to delineate the role of Exp-2 in protein trafficking *in vivo* by targeted gene knock out studies and to identify cargo proteins associated with Exp-2 positive vesicles.

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INACTIVATION OF *PLASMODIUM* AND *BABESIA* PARASITES USING THE S-303 PATHOGEN INACTIVATION SYSTEM FOR RED BLOOD CELLS

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Despite donor screening and testing for specific pathogens, emerging pathogens and organism loads too low to be detected by standard testing methods may result in contamination of red blood cells (RBC) intended for transfusion. A second generation pathogen inactivation (PI) system for RBCs has been developed as a proactive measure to prevent transfusion-transmission of viruses, bacteria and protozoan parasites. This system uses S-303 to crosslink nucleic acids, preventing replication of contaminating pathogens and leukocytes. Glutathione (GSH) is included to quench nonspecific reactions. The system also includes a diluent solution designed to enhance inactivation of pathogens. The aim of this study was to determine the efficacy of inactivation of malaria (*P. falciparum*) and *Babesia* (*B. divergens*) in RBCs by the S-303 PI system. RBCs suspended in AS-5 were prepared from whole blood (450-500 mL) held overnight at ambient temperature and separated without platelet recovery. Four replicate inactivation experiments were performed for each organism. Individual RBC units were used for each replicate. RBC units were inoculated with the organism to be tested and the infected units were then combined with GSH and diluent. A control sample of 5 to 6.6 mL was removed and immediately assayed for viable parasites. S-303 was then added to the RBC mixture resulting in a final concentration of 20mM GSH and 0.2mM S-303. The units were incubated for 3 hours at room temperature before being assayed for residual viable parasites. Preliminary results indicate that *P. falciparum* was inactivated to below the limit of detection and *B. divergens* was inactivated by more than 5 logs. These results indicate that high titers of *Plasmodium* and *Babesia* can be inactivated in RBCs under conditions compatible with blood center operation.

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DETERMINING THE EFFECTS OF *PLASMODIUM FALCIPARUM* MALARIA ON PRIMARY INFECTION OF B CELLS BY EBV: MODELING EARLY EVENTS IN BURKITT'S LYMPHOMAGENESIS

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Endemic Burkitt's lymphoma (eBL), the most common pediatric cancer in sub-Saharan Africa, is an aggressive B cell lymphoma with two infectious cofactors: Epstein Barr virus (EBV) infection and frequent *Plasmodium falciparum* malaria. Recent research suggests that *P. falciparum* parasites increase the lytic activation of EBV from B cell lines. It was shown that *P. falciparum* parasites exert their effect on EBV through polyclonal activation B cells. *Plasmodium falciparum* parasites contain multiple polyclonal B cell activators, including CpG DNA, which activates toll-like receptor 9 (TLR9), and Pf Erythrocyte Membrane Protein-1 (PfEMP1), which binds surface immunoglobulin as well as CD36. It was recently shown that activation of B cells using CpG oligonucleotide sequences increased the infection and EBV-induced proliferation of B cells. We hypothesized that *P. falciparum* parasites increase the primary EBV infection and EBV-induced proliferation of B cells. Our preliminary *in vivo* studies suggest that *P. falciparum* indeed increases these measures. We observed an increased frequency of EBV infected peripheral blood mononuclear cells in children in a high malaria area compared to those in a nearby low malaria area of western Kenya by quantitative PCR. Our ongoing fieldwork in Kisumu, Kenya, aims to determine the effect of acute malaria on B cell phenotypes and EBV carriage within the B cell compartment by flow cytometry. Our *in vitro* studies aim to determine the effects of *P. falciparum* on the establishment of EBV latency. We have shown that TLR9 ligation in human PBMC increases surface expression of the EBV receptor, CD21 on B cells. We also showed that TLR9 ligation synergistically increased the proliferation of B cells in culture by CFSE assay. We have expressed recombinant PfEMP1 in order to determine the effect of the other known malaria B cell activator on EBV infection. Overall this work will help elucidate the interaction of *P. falciparum* and EBV with the goal of discovering preventative strategies for eBL.

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THE GENETIC DIVERSITY OF *PLASMODIUM VIVAX* IN POPULATIONS FROM THE AMERICAS AS INFERRED FROM MITOCHONDRIAL GENOME SEQUENCES

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Plasmodium vivax is the most prevalent human malaria parasite in the Americas. Previous studies have contrasted the genetic diversity observed in populations from the Americas to those in Asia and the Pacific, concluding that the populations in the New World exhibit low genetic diversity likely due to their more recent introduction. Here we investigate the genetic diversity of the *P. vivax* population in the Americas and compare the observed pattern with other populations from around the globe. Overall, the Americas as a region harbors a *P. vivax* genetic diversity that is comparable to that observed in Asia and the Pacific. We observe that there is a distinct and relatively ancient clade of *P. vivax* in South America as well as other lineages that could have resulted from independent introductions. Whereas the finding of an ancient lineage is not per se evidence that it was introduced to the region at the time of that lineage's origin, it does provide evidence of a more complex evolutionary history than previously thought. The emerging pattern indicates a strong geographic population structure that could explain previous views that *P. vivax* in this region has low genetic diversity since several studies were carried out in limited geographic areas. We propose that whereas in Asia human migration increases local genetic diversity, the strong geographic structure evident in the Americas has resulted in patterns of low local genetic diversity but high regional diversity. Such a complex pattern needs to be considered in genetic diversity assessments of genes encoding vaccine candidate antigens.

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ASCERTAINING THE RADIATION OF PRIMATE MALARIAS IN ASIA: USING COMPARATIVE APPROACHES TO UNDERSTAND THE EVOLUTION OF MEROZOITE PROTEINS

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There has been great interest in understanding the origin of the different species that cause human malaria. Here we investigate the evolution of major malarial parasite groups that infect non-human primates. We report the result of biodiversity surveys that have allowed us to discover several lineages of primate malarias in Asia, specifically from macaques and orangutans. Overall, we found that the radiations of primate malarias, including those that infect humans, are the result of a complex evolutionary history that includes several host switches. We also found evidence of lineages that could be new *Plasmodium* species in macaques. Then these species are used in comparative approaches focusing on understanding genes important in the invasion of the red blood cell. These non-human primate malarias from oranguntan and macaques are used to study antigenic proteins involved in the invasion of the red blood cell (AMA-1 and GPI anchored merozoite proteins). Overall, we found that these proteins are under different forms of natural selection. There are lineage specific selective pressures but also some conserved motifs across species indicating strong purifying selection or functional constrains. We conclude that the study of non-human primate malarias provides valuable information to better understand emerging patterns in the genetic diversity of human parasites.

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LIMITATIONS OF SINGLE GENE MITOCHONDRIAL APPROACHES FOR IDENTIFYING SPECIES OF MALARIAL PARASITES

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Ascertaining the evolutionary history of the protozoan genus *Plasmodium*, which includes the agents of human malarias, has been an issue of great interest in both the malaria and evolutionary biology research communities. Such interest has driven several biodiversity assessments of malarial parasites in natural populations of non-human primates and birds. The use of mitochondrial markers in malarial parasites, specifically partial sequences of the gene encoding Cytochrome B (Cyt B), has been promoted as a method for both species identification and for discovering new species. Here we test both the potential and limitations of mitochondrial genes, including complete Cyt B sequences, as biodiversity assessment tools in malarial parasites by analyzing a group of well-characterized *Plasmodium* species and contrasting the results of single mitochondrial gene approaches with those from complete mitochondrial genomes. We found that CytB has strong phylogenetic information that allows the correct differentiation of distantly related parasites such as the four human malarias. However, CytB cannot reliably uncover many recent phylogenetic relationships of species that radiated at a scale of 2-5 million years ago. Our conclusion is that single gene approaches do not contain enough information to build reliable molecular phylogenies or define new species.

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THE EUKARYOTIC PATHOGEN DATABASE RESOURCE (EUPATHDB): A EUKARYOTIC PATHOGEN GENOME RESOURCE

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Bioinformatics studies of eukaryotic pathogens are an important part of developing new drug targets and diagnostics for diseases such as malaria, chagas, sleeping sickness and compromising diarrhea. The Eukaryotic Pathogen Database Resource (http:/eupathdb.org) integrates genome sequence and annotations with functional genomics data in searchable databases for: *Babesia and Theileria* (piroplasmadb.org); *Crithidia*, *Endotrypanum*, *Leishmania*, and *Trypanosoma* (tritrypdb.org); *Cryptosporidium* (cryptodb.org); *Eimeria*, *Gregarina*, *Neospora*, and *Toxoplasma* (toxodb.org); *Encephalitozoon*, *Enterocytozoon*, *Nematocida*, *Nosema*, *Octosporea*, and *Vivraia* (microspoidiadb.org); *Entamoeba* (amoebadb.org); *Giardia* (giardiadb.org); *Plasmodium* (plasmodb.org); and *Trichomonas* (trichdb.org). The specific advantage of the EuPathDB family of databases lies in the breadth of pathogens represented (78 organisms), the intuitive graphic web-interface, the extensive repertoire of pre-built searches, and the search strategy system that brings the power of genomics to novice users. Databases are updated and expanded bimonthly with comprehensive data sets ranging from genome sequence and annotations to gene and protein expression data to field isolates of parasites. Multistep search strategies are built one step at a time choosing from more than 90 pre-built searches, and successive searches can easily be combined in ways that refine the biological meaning of results. Our extensive user-support system includes on-line video tutorials, an email hotline for questions that receive a response within 48 hours, and hands-on workshops at locations worldwide. EuPathDB's user-friendly search strategy system embedded in full and up-to-date databases offers researchers a powerful tool for revealing meaningful biological relationships during computational experiments that support hypothesis-driven research. Attend this talk/poster for an overview of this resource. Hands-on demonstrations and help are available at our booth in the exhibit hall.

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EVALUATION OF THE ANTIMALARIAL ACTIVITY OF KOLAVIRON IN *PLASMODIUM BERGHEI* - INFECTED MICE

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Malaria is a major health problem in the world in general and in Sub-Saharan African countries. However, it is also becoming more difficult to treat malaria due to increasing drug resistance. Therefore, the need for discovery of alternative drugs is urgent and paramount. In order to study the antimalarial activity of Kolaviron (KV), Kolaviron was extracted from powdered seeds of *Garcinia kola* using methanol and chloroform. *Plasmodium berghei* was inoculated into Swiss albino mice. The mice (n=46) were infected with 1 x 10^7 parasites intraperitoneally. The extracts were administered by an intra gastric tube daily for four days commencing after establishment of the parasite in the mice. The control group (n=10) received the same amount of corn oil (vehicle used to dissolve extract) while two groups received respectively 100mg/kg KV and 200mg/kg KV extract (n=10). The remaining two groups received chloroquine (10mg/kg body weight) and arteether (3.2mg/kg/body weight) and served as the standard reference drugs, All drugs were administered through the oral route. All animals were monitored for parasitemia and PCV changes for 7 days. Blood samples and liver homogenate were prepared and used for enzyme assays. Data were compared statistically between the groups and $p<0.05$ was regarded as significant. Result showed that in *P.*

berghei infected mice treated with kolaviron (200mg/kg), the percentage parasitemia decreased significantly ($p<0.05$) compared to untreated control animals. However, there was significant ($p<0.05$) increase in PCV levels of the parasite-infected CQ-treated, AE-treated, 100mg KV-treated and 200mg KV-treated groups post-treatment. However, kolaviron (200mg/kg) has a protective effect against liver and brain damage induced by malaria parasite. Kolaviron exerted antimalarial activity comparable with chloroquine and arteether by decreasing parasitemia and causing increase in PCV that suggests its haemopoetic effect. It is not toxic.

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IDENTIFICATION OF ANTI-MALARIA BIOACTIVE COMPOUNDS FROM NAMIBIAN MEDICINAL PLANTS

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Medicinal plants are used to treat malaria and its symptoms such as febrile illness in rural settings in Namibia where access to health facilities is a challenge. It is important to study the efficacy of these indigenous treatments to validate their use and to investigate them as potential sources of new antimalarial compounds. Resistance and reduced sensitivity to ACTs have been reported, making such research imperative. Namibia is in the pre-elimination phase of malaria and an increased number of febrile illnesses are due to microbial infections. The objectives of this study are to identify anti-malaria compounds from selected Namibian plants and to determine the antimicrobial effect of extracts from medicinal plants. *V. infausta, G. coleosperma, M. sericea, Z. mucronata, A. inflata, O. dregeanum, P. angolensis D. mesipliloformis and Z. marlothii* were selected using secondary sources, survey data and literature review of medicinal plants in Namibia. Plant roots, bark and leaves were harvested and aqueous and organic extracts were prepared for analysis. Thin layer chromatography was used for phytochemical analysis, whilst column chromatography was used to fractionate the plant extracts for bioassays on *P. falciparum* 3D7 culture *in vitro*. The antimalarial compounds will be further analyzed with high performance liquid chromatography (HPLC) and nuclear magnetic resonance (NMR). Qualitative screens for antifungal, antibacterial, protease inhibitor and antioxidant assays were also carried out. Phytochemical analysis showed the presence of antimalarial compounds including, anthraquinones, flavonoids, terpenoids and steroids in the plants; *A. inflata* did not have terpenoids and steroids whilst *O. dregaunum* did not have flavonoids. All the plants showed significant antioxidant activities whilst all the plants exhibit anti-bacterial activities except for *V. infausta.* However, *O. dregeanum* did not show anti-fungal activities. All the plants exhibited protease inhibitory properties. Bioassay guided fractionation, HPLC, MALDI-MS and NMR will be used to identify the antimalarial compounds of interest.

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CHEMOGENOMIC APPROACH TO IDENTIFY AND VALIDATE THE TARGET OF A DIVERSITY-ORIENTED SYNTHESIS PROBE

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The emergence and spread of drug resistance to antimalarial therapies remains a pressing concern with reports of artemisinin-based treatment failures escalating the need for novel antimalarial chemotherapies. The availability of complete genome sequences of different *Plasmodium* species and comparative bioinformatics have divulged several metabolic pathways for antimalarial drug discovery and genome-wide methods for target identification and understanding mechanisms of resistance. We have adopted a chemogenomics approach for identifying highly potent bioactives that can be powerful probes of parasite-specific biological processes. Here we present studies of a novel probe from the Diversity-Oriented Synthesis Informer Set (DOS-IS) library with sub-nanomolar activity against the parasite in phenotypic whole cell assays. The DOS-IS is a representative collection of the >100k DOS compounds that have been synthesized at the Broad Institute. Compounds that are derived for DOS pathways aim to cover chemical space extending beyond the common confinements set by "drug-like" parameters, which is characteristic for traditional MedChem libraries and limits the diversity of represented compounds. We successfully applied an intermittent selection protocol to isolate Dd2 parasites that were 100-fold less sensitive to lead compounds in order to investigate the mode of action and molecular target of the DOS probe. To identify the genetic changes that confer resistance, we employed a whole-genome sequencing approach comparing the resistant mutants to the Dd2 parental line. These studies have led to the identification of target mutations in the Qi site of cytochrome b. The DOS mutants remain fully sensitive to atovaquone, suggesting that cross-resistance to both Qi and Qo site inhibitors might be challenging for the parasite and represent a promising avenue for the development of combination therapies. Further studies to determine the effects of targeting multiple active sites in a single enzyme and the ability of the parasite to develop dual resistance are underway.

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NOVEL SERIES OF TRIAZINES FOR MALARIA TREATMENT AND CHEMOPROPHYLAXIS

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Malaria is the number one infectious disease threat for deployed US troops. There is a critical need for the discovery of new classes of anti-malaria drugs due to the therapeutic challenges emanating from the emergence of resistance in many structural classes of current therapies. A thorough retrospective analysis of Walter Reed Army Institute of Research's compound collection has indentified a substituted triazine series that may be a single dose partner drug with minimal potential for resistance. Robust structure activity relationships (SAR) were established using *in vitro* blood stage assays with >250 analogs, wherein a number of compounds have been shown to possess oral *in vivo* efficacy in a modified Thompson assay. An early lead, exhibits excellent *in vitro* potency, excellent selectivity relative to two mammalian cell lines and complete cures (5/5 mice) as a single 160 mg/kg PO dose highlighting the potential for single dose cures. The series features a short chemical synthesis that will allow for rapid SAR development and a low cost of goods. The profile of this early lead and our approach to develop this series will be presented.

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STUDIES TO ELUCIDATE THE MECHANISMS OF ACTION OF BENZOXABOROLES AGAINST *PLASMODIUM FALCIPARUM*

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There is an urgent need for new antimalarials with novel mechanisms of action. We have identified several series of boron-containing compounds with excellent *in vitro* potency and *in vivo* efficacy (for AN3661 IC_{50} 37 nM against W2-strain *Plasmodium falciparum*; ED90 0.58 mg/kg and 0.3 mg/kg against murine *P. falciparum* and *P. berghei* respectively). We are investigating the mechanisms of action of benzoxaboroles against *P. falciparum*, focusing on inhibition of protein synthesis and of leucyl tRNA synthetase (LeuRS), as leuRS is an antimicrobial target of other benzoxaboroles. AN3661 and 9 other benzoxaboroles of various scaffolds

were tested in assays of stage-specificity, inhibition of protein synthesis, and inhibition of plasmodial leuRS activity. The trophozoite stage was most sensitive to AN3661. 8h incubation of synchronous trophozoites with 370nM AN3661 inhibited parasite development by 80%, compared to 40% for rings and 10% for schizonts. Inhibition of protein synthesis was assessed by comparing the incorporation of 14[C] leucine by parasites treated with test compounds or controls. Inhibition of cytoplasmic and apicoplast LeuRS from *P. falciparum* extracts was studied by including S. cerevisiae and *E. coli* tRNA, respectively in biochemical assays. In preliminary studies a dose-dependent inhibition of protein synthesis and LeuRS activity was observed for two related benzoxaboroles, but not AN3661. We also selected for *P. falciparum* with decreased sensitivity to AN3661 by culturing W2 parasites in increasing concentrations of the compound. Parasites were selected with ~100-fold decreased sensitivity (IC_{50} 4 μM). Interestingly, some of the other benzoxaboroles shared the marked loss of activity against the selected parasites, while others demonstrated only a modest change or no difference in activity between initial and selected parasites, suggesting different mechanisms of action for different compounds. In summary, benzoxaboroles are a promising new class of antimalarial compounds, and preliminary studies suggest more than one antimalarial mechanism of action.

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NO CLINICALLY RELEVANT ADVERSE EVENTS CORRELATED WITH PLASMA ARTESUNATE OR PLASMA DIHYDROARTEMISININ EXPOSURE PARAMETERS FOLLOWING SINGLE- OR MULTIPLE-DOSE OF ADMINISTRATION OF ARTESUNATE, OR ADMINISTRATION OF ARTESUNATE TO PATIENTS WITH UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA

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Artesunate (AS) is an artemisinin derivative which along with its active metabolite dihydroartemisinin (DHA), has activity against all erythrocytic stages of *Plasmodium falciparum* and currently in use as first line treatment in SE Asia against severe malaria. The metabolism of AS involves esterase cleavage in the plasma to DHA. This conversion also occurs via various cytochrome P450 enzymes (most notably CYP2A6). The pharmacokinetic and pharmacodynamic parameters of this drug and its active metabolite were reviewed with data from three previous studies (two safety and tolerability dose ranging phase I studies and one safety and efficacy phase II study in adults with uncomplicated malaria). Pharmacokinetic parameters including concentration of AS at initial injection (AS C_0), AS area under the curve (AS $AUC_{0-\infty}$), DHA maximum concentration (DHA C_{max}), and DHA area under the curve (DHA $AUC_{0\text{-last}}$) were compared to adverse events considered possibly or probably related to the study drug including: neutropenia, leucopenia, hypotension, pyrexia, infusion site pain, bradycardia, vomiting, hemoglobinuria, and anemia. This was done using a logistical regression analysis plot of the parameters and comparing the values to the incidence of the adverse events. No increase in frequency of adverse events was statistically correlated to increased level of any pharmacokinetic parameter measured. Thus concluding that both AS and its active metabolite DHA are equally well tolerated and safe at efficacious doses of parent drug.

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ENANTIOSELECTIVE PHARMACOKINETICS OF PRIMAQUINE BY HEALTHY HUMAN VOLUNTEERS

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Primaquine (PQ), the drug of choice for radical cure of relapsing *Plasmodium vivax* malaria, is currently used as a racemic mixture approximating a 50:50 ratio of (+) and (-) enantiomers. Earlier reports and recent studies have indicated differential therapeutic profiles of PQ enantiomers. Enantioselective pharmacokinetic, pharmacodynamic & pharmacologic characteristics may contribute to differential therapeutic indices of PQ enantiomers. A study was conducted with healthy adult human volunteers (age 26-51 years, with different racial/ethnic backgrounds) to determine plasma PK profile of enantiomers of PQ and carboxyprimaquine (cPQ), the major plasma metabolite. The individuals were orally administered three tablets of primaquine phosphate (equivalent to a total dose of 45 mg primaquine base) (Sanofi-Aventis US) 30 min after a normal breakfast. Blood samples were collected at different time intervals after administration of PQ and plasma samples were analyzed using LC-MS for enantiomers of PQ & cPQ. Plasma PQ levels were low and variable for both parent enantiomers and peaked around 2-4 hrs. Peak (-)-PQ levels ranged from 31-131 ng/mL. Peak (+)-PQ levels ranged from 21-146 ng/mL, around 2-4 hours. cPQ levels were much higher and surprisingly consistent from subject to subject, considering the variability in the parent levels. The peak levels of cPQ were observed at 8 hr (1200 ng/ml). However, very high levels were still present at 24 hr. This is consistent with earlier published studies on PQ pharmacokinetics. The key finding in this study was that essentially all of the cPQ detected was (-)-cPQ. (+)-cPQ was two orders of magnitude lower than (-)-cPQ, and in most samples it was only detected under the limit of quantification. The results suggest a markedly more rapid metabolism of (-)-PQ to (-)-cPQ than (+)-PQ. Alternatively, the (+)-PQ or (+)-cPQ could be rapidly converted to another metabolite(s) or distributed to tissues. This study confirms enantioselective pharmacokinetic and metabolic profiles of PQ and supports further clinical evaluation of PQ enantiomers.

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IN VITRO AND *IN VIVO* ANTIMALARIAL EVALUATION OF TIGECYCLINE IN COMBINATION WITH CHLOROQUINE

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Tigecycline (Tygacil®) is the first clinically available drug in a new class of antibiotics called "Glycylcycline". It is a semi-synthetic derivative of Minocycline with a unique and novel mechanism of action in bacteria. Several antibiotics have shown promising antimalarial effects and may be useful for malarial chemotherapy in combination with standard antimalarial drugs. Recently, tigecycline was tested against culture-adapted strains as well as clinical *Plasmodium falciparum* isolates from Lambaréné, Gabon, as reported previously. Tigecycline was found to act faster against the malaria isolates than any of the other antibiotics tested. Tigecycline was also tested against clinical isolates of *P. falciparum* from Bangladesh as reported previously. These study demonstrate the potential of tetracycline derivatives in the development of improved antimalarials and prompted us to evaluate the antimalarial potential of tigecycline *in vitro* against CQ sensitive and resistant strains of *P. falciparum* and also *in vivo* in *Plasmodium* berghei - mouse malaria model. The antibiotic was also tested

in combination with chloroquine (CQ). Tigecyline was more active against CQ-resistance (CQ-R) W2 strain than CQ-susceptible (CQ-S) D6 strain of *P. falciparum*. The chloroquine & tigecycline combination was selectively synergistic against the CQ-R (W2) strain, while the CQ susceptibility of CQ-S (D6) strain of *P. falciparum* was unaffected in combination with tigecycline. Further, treatment of P. berghei infected mice with Tigecycline (i.p.) caused significant suppression in parasitemia development and prolonged the mean survival time. Tigecycline in combination with suboptimal doses of chloroquine produced complete cure in *P. berghei* infected mice. These results support further evaluation of tigecycline as a potential combination candidate for treatment of drug-resistant cases of malaria.

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HIGH THROUGHPUT/HIGH CONTENT *IN VIVO* SCREENING OF ANTIMALARIAL HITS AS A SOURCE OF INNOVATIVE DRUG DISCOVERY PROGRAMS

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The recent disclosure of thousands of compounds active *in vitro* against the erythrocyte stage of *Plasmodium falciparum* is a major breakthrough for malaria eradication. However, the high number of potential starting points of drug discovery programs challenges the methods available to identify compounds that can lead to antimalarial medicines. In this work we show the feasibility of a new strategy to select efficacious, orally bioavailable and non-toxic compounds as optimal starting points of drug discovery projects based on a *P. berghei* high-content/high-throughput *in vivo* screening assay. This assay was developed by allometric extrapolation of human parameters of efficacy into mice. The assay is robust, minimizes the use of mice per compound (two mice) and allows detecting compounds able to stop parasite replication or to induce parasite killing *in vivo*. Finally, circa 600 compounds selected from the Tres Cantos Antimalarial set (TCAMs) active *in vitro* against *P. falciparum* were tested at 50 mg/Kg per oral route in an assay format that allows the evaluation of hundreds of compounds per month. The rate of compounds with detectable efficacy was about 10 %, of which about 33 % were as efficacious as marketed antimalarials. Our results support that the systematic high-content/high-throughput *in vivo* screening of compounds active *in vitro* against *P. falciparum* is a feasible strategy to rapidly select compounds with efficacy comparable to marketed antimalarials as starting points of drug discovery programs. This new paradigm is expected to accelerate the development of new antimalarial drugs.

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IN VITRO EVALUATION OF COMBINATIONS TO MITIGATE HEMOLYTIC TOXICITY OF PRIMAQUINE

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Primaquine (PQ) is the only drug of choice for radical cure of relapsing *Plasmodium vivax* malaria and also a useful drug combination for malaria prophylaxis & prevention of transmission. However, PQ has limited utility due to its narrow therapeutic index, particularly hemolytic toxicities in G6PD deficient individuals. The reactive redox active metabolites generated through cytochrome P450-dependent pathways are regarded as responsible for hemolytic effects of primaquine and other 8-aminoquinolines. The hemotoxic response of the redox active metabolites of PQ, generated in situ, could be measured by accumulation of methemoglobin, kinetic measurement of increase in oxidative stress and depletion of reduced glutathione (GSH) in a microsomal metabolism-linked hemotoxicity assay. Several drugs, which are known to replenish intracellular levels of reduced thiols and/or protect the cells from oxidants injuries, were tested for mitigation of hemotoxic effects of potential toxic metabolites of PQ. N-Acetyl cysteine (NAC) has been reported to produce increase in intracellular GSH, decrease in oxidative stress and increase in erythropoietin (EPO) production. NAC was observed to partially attenuate the hemotoxic effects of 5-hydoxy PQ, a potential hemotoxic metabolite. NAC prevented the 5-HPQ induced accumulation of methemoglobin and oxidative stress and also protected the G6PD deficient erythrocytes from depletion of GSH. Lipoic acid and ascorbic acid also prevented 5-HPQ induced methemoglobin accumulation but did not protect the G6PD deficient RBCs from depletion of GSH. Some CYP inhibitors were also tested for attenuation of hemotoxic response of PQ in a microsomal metabolism linked hemotoxicity assay. The CYP3A4 and CYP2D6 inhibitors produced only partial attenuation. Chloroquine, which has earlier been reported to potentiate efficacy of PQ did not show any effect on PQ induced hemotoxic response *in vitro*. This study may help in developing a rational approach for developing suitable combinations for improving therapeutic utility of primaquine and other 8-aminoquinolines.

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DEVELOPMENT OF A NOVEL CHEMICAL SERIES WITH ACTIVITY AGAINST BOTH BLOOD- AND LIVER-STAGES OF *PLASMODIUM FALCIPARUM*

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Recent progress toward the development of a novel compound series with promising *in vitro* efficacy against both blood- and liver-stage *Plasmodium falciparum* will be described. Following the failure of one such compound to cure malaria-infected mice, focus has been to enhance the pharmaceutical properties of the compound series. Newer analogs, incorporating structural changes that enhance these properties, have been prepared. The *in vitro* efficacies and pharmaceutical properties of these will be described, including the kinetics of *in vitro* microsomal degradation, and the subsequent identification of predicted metabolites.

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MALARIA ELIMINATION IN THE PHILIPPINES: HOW MUCH DOES IT COST?

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Although numerous countries around the world are considering moving toward malaria elimination, there is little guidance as to how to make this transition and what resources would be needed. This study aims to (1) estimate the costs associated with sub-national malaria control programs as they progressively eliminate malaria over time and (2) understand how different environmental and organizational factors influence those costs. The Philippines offers a unique opportunity for conducting an expenditure study on malaria elimination because of its variety of epidemiological and ecological settings, and devolved health system. The country has also adopted a progressive, sub-national malaria elimination strategy unlike national-level strategies of other eliminating countries in the region.

Through key informant interviews and archival record retrieval in five select provinces - Apayao, Benguet, Cavite, Laguna, and Sorsogon_we collect all expenditures associated with provincial malaria elimination programs for at least two years in each province (select years in 1998/99 and 2006/07, based on data availability) to enable comparisons across provinces, across years, and across program phases. Costs are gathered from a program perspective and include contributions (cash, in-kind) from the national government, donors, NGOs, and other private sector partners, but exclude private household expenditures. Preliminary results indicate that overall expenditures per population at risk decrease as programs progress from elimination to prevention of reintroduction, are generally higher when financed with international donor funding, and may be related to the level of economic development within a province. Ongoing data collection seeks to validate and complete these expenditure data as well as gather contextual information to understand these relationships in more depth. Lessons learned from the Philippines' malaria elimination efforts would fill a much-needed gap in information about what the strategies, interventions and financing requirements are to successfully eliminate malaria.

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A CLUSTER-RANDOMIZED TRIAL TO DETERMINE THE IMPACT OF HOTSPOT-TARGETED INTERVENTIONS ON MALARIA TRANSMISSION

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Malaria transmission is highly heterogeneous with some households being disproportionally exposed to malaria-infected mosquitoes. These households experience the highest malaria burden and are also hypothesized to contribute disproportionally to onward malaria transmission. Interventions targeted to these hotspots of malaria transmission may be an effective way to reduce malaria transmission at community level. We aimed to determine this community-wide effect of hotspot targeted interventions in a cluster-randomized trial in Rachuonyo, western Kenya. Hotspots were defined as areas for which there was strong evidence ($p<0.05$) of an elevated prevalence and density of combined AMA-1 and MSP-1 antimalarial antibodies. The presence of these hotspots was confirmed by parasite prevalence PCR. In the period preceding the transmission season of 2012, hotspots were targeted with mass distribution of long lasting insecticide treated nets, indoor residual spraying, larviciding and a focal screen and treat campaign where a sentinel age-group was screened for parasites and the entire household of parasite-positive individuals was treated with a curative dose of antimalarials. The intervention is being evaluated during cross-sectional surveys conducted in the transmission season (June-September). The methodology of the intervention will be presented, together with an analysis plan to estimate the extent of the community impact of hotspot-targeted interventions. Results from a first evaluation surveys will be presented.

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EXAMINING THE IMPACT OF COMBINING MULTIPLE INTERVENTIONS FOR MALARIA CONTROL BY USING A SPATIAL AGENT-BASED MODEL

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Malaria is a priority public health problem today. To reduce the disease burden, an essential objective is the scale-up application of proven, evidence-based, mosquito control interventions. The selection of interventions should consider local epidemiological parameters, some of which may vary greatly. For example, insecticide-treated nets (ITN/LLIN) need the development of community-based distribution strategies seeking high coverage. Indoor residual spraying (IRS) is more appropriate in areas of unstable or epidemic transmission (e.g. in the eastern African highlands), and usually requires detailed reconnaissance of the target area. Source reduction may be applicable in especially arid areas, with a limited number of clearly defined breeding sites (e.g. in parts of the Horn of Africa). In some cases, even a single method of intervention may impact the transmission on multiple fronts. A recent study shows that multiple vector management scenarios, in combination, are 100 fold more effective in reducing transmission than any single measure used in isolation. Recently, much interest is observed on using multiple interventions in unison, with the expectation of achieving a synergistic effect caused by them. However, more detailed studies are required to analyze the efficacy of potential combinations of interventions before actually carrying out the more costly and time-consuming empirical evaluations. We have developed a spatial agent-based model (ABM) of malaria that simulates the vector dynamics lifecycle, including various stages of the dominant vector species *Anopheles* Gambiae. The ABM provides opportunities for more realistic modeling of important spatial events, such as bloodmeal seeking and oviposition. We also developed a landscape simulator to simulate landscapes used by the mosquito vectors. Using the spatial ABM and the landscape simulator simultaneously, we can investigate the impacts of multiple interventions for malaria control, and select the optimum mix of interventions for specific locations of interest. For example, initial results of our model show that carefully selected combinations of source reduction and ITNs yield better impacts than either of these interventions applied alone. The overall goal of our modeling effort is to quantitatively measure the effectiveness of multiple interventions and to demonstrate the model's ability to discover the synergistic benefits of using them.

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MALARIA CONTROL AND ELIMINATION IN SRI LANKA: DOCUMENTING PROGRESS AND SUCCESS FACTORS IN A CONFLICT SETTING

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As malaria transmission declines and malaria programs shift their focus from malaria control to elimination, it is vital to have documentation of the strategies that countries have used and are currently applying as they seek to eliminate malaria. Our case study of Sri Lanka, which has a long history of malaria control, including a period of near elimination and resurgence in the 1960s, aims to capture the key factors behind the country's decline in malaria over the last decade. The case study employed qualitative and quantitative methods, using data triangulation to compare and contrast trends. A literature review was conducted, and district and national data were collected on incidence, surveillance and vector control. Trends were observed across years and districts, in particular comparing conflict and non-conflict districts. Thirty-three key informant interviews were conducted. Expenditures in two districts for two years were compiled to identify changes in expenditure. Malaria incidence in Sri Lanka has declined by 99.9% since 1999. During this time, there were major increases in the proportion of malaria infections due to *Plasmodium vivax*, and those occurring in adult males. New vector control strategies were introduced, such as spatial insecticide rotation and long-lasting insecticide-treated nets. A strong passive case detection system is the foundation for diagnosis, while active case detection grew from identifying 1.1% of all infections in 2000 to 13.1% in 2007. Vector control and surveillance measures were maintained in conflict areas. For example, coverage of indoor residual spraying of risk populations in conflict districts was 45.9%

in 2005 (10.9% in non-conflict districts). One of two districts in the study reported a 48% decline in malaria programme expenditure per person at risk from 2004 to 2009, and a decline in prevention costs and an increase in surveillance costs. Malaria is now at low levels in Sri Lanka - 124 indigenous cases were found in 2011. Evidence-driven policy and an ability to adapt to new challenges contributed to this decline.

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EVALUATION OF THE EFFICACY OF IFAKARA ODOUR-BAITED STATIONS AGAINST MALARIA TRANSMISSION IN SOUTHERN TANZANIA: RESULTS FROM BASELINE MOSQUITO SURVEILLANCE

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Large-scale implementation of indoor residual spraying (IRS) and long lasting insecticide nets (LLINs) have significantly decreased malaria transmission across Africa. However, elimination remains elusive since interventions target mainly indoor transmission; thus the need for complementary outdoor interventions. Here we present baseline mosquito-surveillance data from a study designed to assess community-level effects of the Ifakara odour-baited station, a new outdoor intervention that lures and kills malaria mosquitoes.From a population of 2433 households in 3 villages (Kivukoni, Minepa and Mavimba) in southern Tanzania, 1600 households were randomly selected and spatially assigned, based on latitudes, to 16 clusters each consisting of 100 households. Monthly mosquito collections were performed using CDC-Light traps inside 6 households randomly selected from each cluster. The mosquitoes were sorted by taxa and abdominal status, after which a sub-sample of the malaria vectors were examined by (PCR) to distinguish between sibling species. The vectors were also examined by (ELISA) to detect *Plasmodium* sporozoites in their salivary glands. A total of 9,549 An. gambiae s.l and 2,529 An. funestus s.l were collected in the 3 villages during the first 5 months of the survey (Nov-2011 to March-2012). The distribution of An. gambiae s.l and An. funestus was spatially clustered, mostly in a set of adjoining clusters centered around the middle of the study area. At least 75% of An. gambiae s.l and 86% of An. funestus, were collected in adjoining clusters 7 to14, centered in Minepa village. PCR and ELISA analyses are yet to be completed.These preliminary results show that most of the malaria vectors were collected from a set of contiguous clusters in an area centered in Minepa village, suggesting suitability of spatially targeted intervention. Further assessments are underway to determine risk factors associated with this distribution pattern and mosquito house entry, prior to introduction of the Ifakara odor-baited station.

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SERA FROM *PLASMODIUM VIVAX* PATIENTS BOOSTS *P. FALCIPARUM* GAMETOCYTE PRODUCTION *IN VITRO*

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We have previously described higher rates of gametocyte carriage in Cambodian patients who are found to relapse with *Plasmodium vivax* following treatment for *P. falciparum*. This led to the hypothesis that *vivax* coinfection boosts gametocyte production in patients with *falciparum* malaria. We sought to test this hypothesis *in vitro* by comparing gametocyte production in *P. falciparum* erythrocytic cultures grown in media containing sera from *P. vivax* patients vs. pooled naïve sera. NF54 *falciparum* parasites from the same culture flask were inoculated into microtiter wells and maintained with daily media changes containing 30% sera from either Peruvian individuals acutely infected with *P. vivax* or control sera from malaria-naïve donors. Daily blood smears were examined for parasitemia and the development of early stage gametocytes. Over the 4 days of culture, parasitemia among the cultures grown in sera from *vivax* patients was slightly decreased compared to the control. On the other hand, gametocytogenesis occurred at a faster rate in the cultures exposed to *vivax* sera, with a significant difference in proportion of gametocytes by Day 5. At Day 5, in those cultures exposed to *vivax* sera, 37% of parasites were early stage gametocytes (range 22% to 78%) vs. 11% in the control cultures (range 9% to 14%) (p=0.03). A similar trend was seen in cultures exposed to 20% sera from *vivax*-infected patients. Similar results with sera from Cambodian *vivax* patients will be presented, with support from expression data using real-time reverse transcriptase PCR targeting the early-stage gametocyte antigen, Pfg377. If these *in vitro* findings reflect the *in vivo* environment within hosts with mixed *P. falciparum/P. vivax* infection, the implications are that *vivax* coinfection may facilitate transmission of *falciparum* malaria in settings where the species are coendemic and improved control of *P. vivax* may also aid *P. falciparum* control efforts.

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THE FEASIBILITY OF MALARIA ELIMINATION IN SOUTH AFRICA

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Although malaria is still regarded as one of the most important public health burden on the African continent, in South Africa the disease is on the brink of elimination. The last major South African malaria epidemic occurred in 2000 where over 64 000 cases and approximately 500 deaths were reported. During this epidemic most of the cases and death were reported in KwaZulu-Natal, while Limpopo Province accounted for the lowest number of malaria cases and deaths. A decade on, the national number of malaria cases declined to less than 5000 with 44 reported malaria related deaths. Over this ten year period a change in malaria epidemiology was noted. Limpopo Province, adjacent to Zimbabwe, now accounts for most of the malaria cases and KwaZulu-Natal the least. This study involved a retrospective analysis of malaria case and mortality data collected by the malaria information system from 2000 until 2011, with the aim of determining of whether South Africa in the elimination phase of malaria. Our analysis revealed that two of the three malaria endemic provinces, namely KwaZulu-Natal and Mpumalanga Province have achieved the elimination phase cut-off threshold of <1 case per 1000 population. However, Limpopo Province is still in the consolidation phase of malaria control with an incidence of 1.68 cases per 1000 population. These results suggest that South Africa as a country is not eligible for elimination. However, if the malarious areas are partitioned according to their provincial status then both KwaZulu-Natal and Mpumalanga are well into the elimination phase and could eliminate malaria by 2018 as anticipated by the National Department of Health. Unfortunately due to the sustained high levels of malaria transmission in Limpopo Province it is still in the control phase. Data from this study suggests elimination certification for South Africa should be done at a lower level, ideally provincial, rather than at a national level. Moving forward the provincial malaria control programmes need to conclusively determine whether malaria cases are local or imported as well as identify and reduce local foci of transmission. It is also essential that cross-border initiatives are strengthening thereby decreasing imported malaria and local transmission.

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COMMUNITY PERSPECTIVES ON OUTDOOR MALARIA TRANSMISSION AND ITS CONTROL IN RURAL TANZANIA

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Despite the extensive use of indoor residual spraying (IRS) and insecticide treated nets (ITNs), residual malaria transmission persists in many parts of

Africa, partly driven by mosquitoes that bite people outdoors. Outdoor mosquito control devices such as mosquito traps have been proposed as complementary outdoor interventions. Even though outdoor mosquito control devices are clearly worth pursuing, it is essential to also consider the perspectives of a typical user- so as to ensure its sustainability. We assessed the views and behaviors of rural and peri-urban communities in southern Tanzania, regarding outdoor mosquito bites and malaria prevention. A qualitative cross sectional study was conducted in two rural villages (Lupiro and Minepa and one peri-urban village (Lipangalala) located within the Kilombero Valley in southern Tanzania, using semi-structured interviews and structured observation. A total of 30 participants were randomly selected for interview and their houses were observed. A prototype outdoor mosquito control device, was used to assess responses towards such outdoor interventions. Focal areas included a) whether malaria mosquitoes also bite outdoors and whether transmission can also occur and b) means of protection from mosquito bites while they outdoors. Preliminary results suggest that whereas people in these study area appreciate outdoor mosquito bites, most of them still believe that transmission mainly occurs indoors and at midnight. We observed numerous outdoor activities e.g. playing, fetching water storytelling, cooking, shopping and socializing at times when local malaria vectors are known to be most active outdoors. These are risk factors associated with outdoor malaria transmission. Overall, the communities were willing to use and contribute towards financing of the outdoor devices to control malaria. Further analysis is underway and results will be presented at the meeting. Providing well developed outdoor mosquito control devices that are acceptable will contribute substantially in reducing malaria transmission

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VALIDATION AND OPTIMIZATION OF NEW COMMUNITY BASED METHOD FOR IDENTIFYING SUITABLE AREAS TO LOCATE OUTDOOR MOSQUITO CONTROL DEVICES IN SOUTHERN TANZANIA

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Outdoor devices for luring and killing malaria vectors have been proposed as potential complementary interventions alongside existing insecticide treated nets (ITNs) and house spraying with residual insecticides (IRS). To enhance effectiveness of such outdoor interventions, it is essential to optimally locate them in such a way that they target most of the outdoor mosquitoes. We conducted a study to identifying suitable areas to locate outdoor mosquito control devices (Ifakara odor baited stations) one of the outdoor device developed at Ifakara Health Institute.The study was conducted in three villages (Kivukoni, Minepa and Mavimba) in Southern Tanzania. Maps of the villages were produced and sub divided into square grids (200m x 200m). In each village an area covering 300 grids was delineated for study. Using the village gridded maps and GPS to locate and mark all 300 sampling points per village. A list of random unique codes of all the grids (1 to 300) was produced. The trapping devices will be run for 12 hours during the night. In each village sample 300 grids every month and this processes replicate continuously over twelve (12) months so as to cover both dry and rainy seasons. Community meeting in the three villages was conducted where focus group discussion on mosquito life cycle, distributions and malaria transmission was conducted. Participants were provided with a gridded village map and pen, then were asked to rank all the grids by indicating on the grid, the likelihood (on a scale of 1-5) based on their own experiences and knowledge. Five being high number, followed by 4, 3, 2 and one being lowest number of mosquitoes. The data obtained will be imported to ArcGIS 9.3 standard Desktop and display using the ArcMap application. Overall, the communities know where the mosquitoes are abundant. Further analysis is underway and results will be presented at the meeting. Optimal location of developed outdoor mosquito control devices will significantly reduce mosquito biting and outdoor malaria transmission.

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IDENTIFYING AND CHARACTERIZING FOCI OF MALARIA TRANSMISSION IN POTENTIAL MALARIA ELIMINATION SETTINGS IN RURAL SENEGAL

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Sparse distribution of malaria morbidity has become a consistent feature of the disease, particularly in the new context of malaria decline. Foci of residual transmission (hot spots) are particularly relevant to malaria control programme in countries such as Senegal with low transmission intensity, since their presence makes it very difficult to eliminate the disease. Based on the 2008 and 2009 general changes on the epidemiological profile of the disease and in particular on the very low incidence rates observed all over the sub-region (figure), we designed this project to identify and characterize the foci of residual transmission and to pilot methods for surveillance that could be used as part of an elimination programme. Scientific research questions were therefore the following: 1. Has transmission stopped in some areas? a) Evaluation of the validity of health facilities records b) Use of serology to determine if transmission of malaria has stopped in defined area. 2. Why is transmission persisting in some places? Several factors could explain the patchy distribution of malaria (use of preventive measures, housing, environmental or climatic factors. Several activities were conducted to answer to the research questions listed above among which: a) A collection and analyses and validity assessment of 2008-2011 health facilities records. b) A case-control study with a community survey around all cases and controls intervention coverage's in the vicinity of where the cases and the controls live. c) Cross sectional surveys to assess level of malaria transmission from serology d) An environmental evaluation in and in areas with low transmission Data analysis is on going and results will be presented at the conference.

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THE USE OF LOT QUALITY ASSURANCE SAMPLING (LQAS) TO ASSESS MALARIA PARASITE PREVALENCE AND GUIDE MASS SCREENING AND TREATMENT CAMPAIGNS

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As malaria parasite prevalence declines, the utility of standard probability household surveys to measure malaria parasite prevalence decreases for ascertaining relatively precise point estimates. Other survey methods are needed to evaluate malaria interventions and provide estimates of the malaria burden once the prevalence has decreased below 10%. One alternative is lot quality assurance sampling (LQAS), which has been used to define areas as low or high depending upon a threshold set by the surveyor. LQAS has been adapted to the health sector in low and middle income countries as a tool to identify areas with adequate health services of various types, including an application in estimating areas of low and high malaria parasite prevalence. It has the potential to be a useful tool in identifying villages as low and high malaria parasite prevalence, as well as identifying areas of low and high vector control coverage. We used census data from a mass malaria screening and treatment campaign in Southern Zambia to assess the feasibility of using LQAS to identify villages with low malaria parasite prevalence (malaria parasite prevalence <10%) and high ITN coverage (>50% of households within a catchment area owning at least 1 ITN). We ran simulations using the census data to ascertain the sensitivity and specificity of LQAS sampling for randomly

selected individuals and households to detect villages with low malaria parasite prevalence and high ITN coverage respectively. Preliminary results suggest that LQAS is both sensitive and specific at detecting areas with low malaria parasite prevalence and high ITN coverage, and that gains in sensitivity and specificity can be made by sampling individuals 6-18 years of age when classifying villages as low malaria prevalence. It is envisioned that at the outset of dry season malaria screening campaigns, in areas with heterogeneous levels of malaria transmission, LQAS can be employed at local levels to assist classification of catchment areas into mass or more focal screen and treat activities.

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EXAMINATION OF SURVEILLANCE DATA MILESTONES AS ZANZIBAR TRANSITIONS TO THE PRE-ELIMINATION OF MALARIA, 2008-2011

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Zanzibar introduced artemisinin-based combination therapy and intermittent preventive treatment of malaria for pregnant women in 2003 and 2004, respectively, followed by insecticide treated bednets for pregnant women and children under 5 years of age in 2005, and indoor residual spraying of households in 2006. This comprehensive package of interventions has led to *Plasmodium falciparum* prevalence estimates <1% in population-based household surveys since 2008. Specific surveillance indicators with milestones are provided by the World Health Organization to guide programmatic transition from malaria control to pre-elimination to elimination. Transition from malaria control to pre-elimination occurs once the slide or rapid diagnostic test (RDT) positivity rate is consistently <5% among febrile patients. Criteria for transition to the elimination phase includes case reporting from all health facilities and malaria incidence reduced to <1 case per 1000 persons at risk per year. The Zanzibar Malaria Control Programme has collected and analyzed weekly malaria surveillance data from all 142 government health facilities since 2008. Approximately 90% of these facilities use a *P. falciparum* histidine-rich protein-2 based RDT to confirm presence of malaria in febrile outpatients. Between 2008 and 2011 the number of diagnostic tests performed on outpatients at all 142 health facilities increased 130% (from 115,361 to 265,403 tests). The annual test positivity rate among febrile outpatients declined from 3.5% in 2008 to 1.2% in 2011. The weekly test positivity rate exceeded 5% on four separate occasions (10 weeks total) in 2008, but from January 2009 through April 2012 the 5% threshold was exceeded on only four occasions (seven weeks total). Annual malaria incidence among the entire population (1.3 million in 2011) declined from 3.3/1000/yr (95% confidence interval [CI], 3.2-3.4/1000/yr) to 2.4/1000/yr (95% CI, 2.3-2.5/1000/yr) between 2008 and 2011, respectively (27% reduction). Zanzibar is nearing the pre-elimination phase of malaria control, particularly during 2009-11 when the weekly test positivity rate rarely exceeded 5%. Annual malaria incidence in Zanzibar remains 2-3 fold higher than required for transition to the elimination phase.

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AFRICAN WOMEN'S PERIPHERAL BLOOD MONONUCLEAR CELL COMPOSITION IS ALTERED BY *PLASMODIUM FALCIPARUM* INFECTION INDEPENDENTLY OF GESTATIONAL AGE OR DELIVERY

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Placental malaria is caused by sequestration of *Plasmodium falciparum* infected erythrocytes in the intervillous spaces of the placenta, resulting in pathological alterations. There are, however, limited data concerning the profiles of immune cells associated with malaria physiopathology during pregnancy. As part of the STOPPAM study described here, we performed *ex vivo* assays using flow cytometry to evaluate the impact of pregnancy-associated malaria (PAM) on the phenotypic composition and activation status of peripheral blood mononuclear cells (PBMC) and to identify PBMC profiles that are associated with malaria outcomes. STOPPAM was a longitudinal, prospective study conducted in Benin and Tanzania, in which 1000 pregnant women with a gestational age below 24 weeks, were enrolled at each site and followed up until delivery Infected women were identified through examination of thick and thin blood smears. The phenotypes and activation status of PBMC were analysed in samples of subgroups of 131 women (62 infected and 69 uninfected) at inclusion and 111 women (37 infected, 27 'exposed' and 47 uninfected) at delivery in the Benin cohort. Verification of the findings generated by these analyses was sought through identical analyses of PBMC in subgroups of women from the Tanzanian cohort. At inclusion, the frequencies of B cells and their expression levels of the activation marker CD86 were higher ($p=0.04$; $p=0.01$), whilst other parameters such as the expression of HLA-DR on antigen-presenting cells (monocytes, dendritic cells) and the frequency of regulatory T cells were lower ($p=0.01$) in PBMC of infected versus uninfected women. In *P. falciparum*-infected women, anaemia was associated with a decreased frequency of peripheral blood monocytes. At delivery, PBMC of uninfected women had fewer low expressing $CD86^+$ B cells, more plasmacytoïd (p)DC, more myeloïd (m)DC expressing high levels of HLA-DR, and fewer T effector cells ($CD4^+CD25^+CD127^+$) compared to those with infection with respective *p-values* ($p=0.03$, $p=0.01$, $p=0.008$, $p=0.02$ and $p=0.007$). The differences in the PBMC profiles at inclusion compared with delivery will be discussed in the context of possible differences in the duration of the *P. falciparum* infections detected at the two time-points.

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CONSEQUENCES OF MALARIA DURING PREGNANCY ON NEONATAL ANTIGEN PRESENTING CELL ACTIVATION VIA TOLL-LIKE RECEPTOR LIGAND AND ON *PLASMODIUM FALCIPARUM* ANTIGENS RESPONSES IN BENIN

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During pregnancy and early childhood, there is an increased susceptibility to malaria due to parasite-induced modulation of pro-inflammatory responses. The development of a protective immune response requires correct function of Toll-like receptors (TLR) expressed by antigen-presenting cells (APC). TLR activation induces cytokine production and expression of co-stimulatory molecules to trigger antigen presentation to T cells. In newborns, stimulation of APC via their TLR is involved in the progressive development of immune responses during the first months of life and our hypothesis is that pregnancy-associated *Plasmodium falciparum*-malaria may adversely affect this development. The study is part of the STOPPAM project conducted in Benin; a clinical and parasitological follow-up of 200 pregnant women and their children followed from birth to 12 months. To assess the impact of malaria on neonatal immunity peripheral whole blood of children (0, 3, 6 and 12 months) was stimulated either by TLR ligands (polyI:C, LPS, R848, CpGODN) that have distinct effects on APC subsets. Concentrations of pro- and anti-inflammatory cytokines were then evaluated in culture supernatants to investigate activation levels of APC. We performed the stimulations and cytokine assays on blood from 134 newborns from mothers with different malaria histories during pregnancy in order to evaluate the impact of an *in utero* contact with *P. falciparum* antigen on the newborn immune system. As a general trend, the responses of APC to TLR stimulation increased with age. According to a multivariate analysis, the mother's infection at delivery had a significant effect on infant immune responses: higher concentrations of IL6 in response to CpGODN and polyI.C at birth; of IL-10 in response to CpGODN and polyI.C at 3 and 12 month; of TNF-α in response to CpGODN, polyI.C and R848 at 6 months. Our findings suggest that mother's malaria infection at delivery altered the neonatal innate immune responses via TLR activation that may have consequences in the development of immune responses.

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ANTIGEN-SPECIFIC CYTOKINE RESPONSES TO *PLASMODIUM FALCIPARUM* DURING A PERIOD OF SUSTAINED LOW MALARIA TRANSMISSION IN A HIGHLAND AREA OF KENYA

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Acquisition and maintenance of protective immunity to malaria relies on the frequency of exposure. Despite the global scale-up of malaria control that has realized a reduction in transmission, the longevity of antigen-specific cellular immune responses to *Plasmodium falciparum* in areas of very low transmission is not well characterized. In addition, most studies assessed responses to one or two cytokines and not to multiple cytokines that may be involved in protection from *P. falciparum* infection or disease. We assessed levels of pro-and anti-inflammatory cytokines in response to the *P. falciparum* pre-erythrocytic (CSP, LSA-1, TRAP) and blood-stage (AMA-1, MB2, MSP-1) antigens in 100 individuals from a highland area of western Kenya with unstable malaria transmission. Responses were assessed from April 2008- April 2009, following a 14-month period from March 2007- April 2008 during which no malaria transmission was observed. Malaria incidence during this period was very low, with an incidence of 20%) of responses to most *P. falciparum* antigens were seen for IFN-γ, RANTES and TNF-α, whereas lower frequencies of responses (<20%) to most *P. falciparum* antigens were seen for the IL5, IL-6 and IL-10. Three distinct patterns of immune responses to *P. falciparum* antigens emerged, with some variation by antigen: a significant decrease in immune response during the first 6 months that was sustained at 12 month follow-up (IFN-γ, TNF-α and IL-10), a significant decrease during the first 6 months, followed by a modest increase at 12 month follow-up (IL-5), and no change over time (IL-6, RANTES). Patterns did not differ between pre-erythrocytic and blood-stage antigens. In the absence of high-level malaria transmission, IFN-γ, TNF-α, IL-5 and IL-10 responses to *P. falciparum* decrease, while IL-6 and RANTES responses are stable. Future studies will assess whether loss of specific immune responses is associated with loss of clinical immunity to malaria.

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PF332-C231-REACTIVE ANTIBODIES AFFECT GROWTH AND DEVELOPMENT OF INTRA-ERYTHROCYTIC *PLASMODIUM FALCIPARUM* PARASITES

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The *Plasmodium falciparum* antigen 332 (Pf332), is a megadalton parasite protein expressed at the surface of infected red cells during later stages of the parasite's developmental cycle. Antibodies to different parts of this antigen have been shown to inhibit parasite growth and adherence to host cells with or without ancillary cells. However, the mechanisms involved in these inhibitions remain largely unknown. We further analysed the activities of specific antibodies with regard to their specific mechanisms of action. For these analyses, affinity purified human antibodies against epitopes in the C-terminal fragment of Pf332 (Pf332-C231) were employed. All purified antibodies recognized Pf332-C231 both by immunofluorescence and ELISA. IgG was the main antibody isotype detected, although all sera investigated had varying proportions of IgG and IgM content. All the antibodies showed a capacity to inhibit parasite growth in *P. falciparum* cultures to different extents, mainly by acting on the more mature parasite stages. Morphological analysis revealed the antibody effects to be characterized by the presence of a high proportion of abnormal schizonts (15-30%) and pyknotic parasites. There was also an apparent antibody effect on the red cell integrity, as many developing parasites (up to 10% of trophozoites and schizonts) were extracellular. In some cases, the infected red cells appeared to be disintegrating/fading, staining paler than surrounding infected and uninfected cells. Antigen reversal of inhibition confirmed that these inhibitions were antigen specific. Furthermore, the growth of parasites after 22-42 h exposure to antibodies was investigated. Following the removal of antibody pressure, a decreased growth rate of these parasites was seen compared to that of control parasites. The present study confirms the potential of Pf332 as a target antigen for parasite neutralizing antibodies, and further indicates that epitopes within the C231 region of Pf332 should constitute important tools in the dissection of the role of Pf332 in the biology of the malaria parasite, as well as in the design of a malaria vaccine.

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LONGITUDINAL DYNAMICS OF FUNCTIONAL AND SEROLOGIC ANTI-MALARIA ANTIBODY RESPONSES IN KENYAN INFANTS

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Maternal antibodies transferred to the fetus during pregnancy protect the infant from malaria infection. These antibodies are thought to wane by 6-9 months of age after which infants slowly generate their own anti-malaria antibodies in response to repeated infections. In this study we measured anti-malaria antibodies in plasma samples from a longitudinal cohort of infants from 2006-2008 (Kisumu District, Kenya) with blood samples drawn approximately every month from birth to 30 months of age. Plasma samples from 22 infants (~17 blood samples/infant) were examined for the presence and magnitude of anti-malaria antibodies by a) serology to 11 malaria antigens, b) human antibody recognition of variant surface antigens exported to the erythrocyte membrane from 4 different parasite strains, and c) functional antibody-mediated growth inhibition of cultured parasites. Differential patterns of anti-malaria antibody waning and waxing were observed. Serologic measured antibodies directed against the majority of antigens tested (AMA1, MSP1, EBA140, EBA175, EBA181, LSA1, PfCSP, PfCelTOS) waned by 6-12 months of age. For all antigens tested except EBA175, infant antibody levels and prevalence reached or exceeded birth levels by 18-32 months of age. The most robust responses were observed with AMA1 and LSA1. Growth inhibitory antibodies were measured from birth to 12 months of age and observed at low levels throughout this time course which peaked between 6 and 9 months of age. Antibodies directed against variant surface antigens reached a nadir at 12 months and slowly climbed from 12-30 months of age. Infant variant surface antibody levels and prevalence did not reach birth levels. Infants with evidence of recent malaria infection (defined by positive blood smear, PCR or IgM responses to more than 5 antigens) had some evidence of boosting of antibodies directed against variant surface antigens, but not serologically measured anti-malaria antibodies. Whether these antibodies are markers of exposure or protection is not clear.

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RAPID DEPLETION OF B AND T LYMPHOCYTES DURING *PLASMODIUM YOELII* 17XNL MURINE MALARIA IS ACCOMPANIED BY A SELECTIVE GAIN IN ACTIVATED CD69$^+$ CD4$^+$ AND CD8$^+$ T CELLS AND ANTIBODY SYNTHESIZING CD19$^+$CD138$^+$ PLASMABLASTS

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To better understand the mechanisms operational during the formation of malaria immunity, we constructed a detailed immunological profile throughout the course of a *Plasmodium yoelii* 17XNL infection. At peak parasitemia (day 9), infection was marked by a rapid depletion of TCR$\alpha\beta^+$ T cells. There was a 50% decrease ($p<0.0001$) in the frequency of both CD4$^+$ and CD8$^+$ T cells compared to uninfected mice. However, despite this apparent loss, there was a significant increase ($P<0.0001$) in activated (CD69$^+$) CD4$^+$ and CD8$^+$ T cells indicating that this loss could be due to activation induced cell death (AICD) - a well defined mechanism of cell death in which activation through the T cell receptor results in apoptosis. There was also a 35% decrease in CD19$^+$ B cells at peak parasitemia compared to uninfected mice. Despite this substantial loss in B cells, there was a significant increase in the frequency of antibody synthesizing CD19$^+$CD138$^+$ plasmablasts that was maximal (9.2%) at peak parasitemia. A proinflammatory response, marked by the presence of IFN-γ^+CD4$^+$ T cells and the IFN-γ, IL12p70 and KC cytokines, was observed during the ascending phase (day 6) of infection. In contrast, an anti-inflammatory response, characterized by production of the IL-5, IL-6 and IL-13 cytokines, was induced during the clearance phase of infection (day 13). Finally, maximal levels of TCR$\gamma\delta^+$ T cells (6.8%) and Ly6g$^+$ neutrophils (2.8%) were observed at day 13, indicating a possible role for these cells in parasite clearance. Ongoing studies are directed to define the antigen specificity for the loss of splenic T and B cells and to understand the mechanism of the switch from a Th1 to Th2 response

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INDIVIDUAL AND EPISTATIC EFFECTS OF GENETIC POLYMORPHISMS OF CD40, CD40L AND BLYS GENES, CO-STIMULATORY MOLECULES ON SUSCEPTIBILITY TO *PLASMODIUM VIVAX* MALARIA

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Following the candidate gene approach we analyzed the *CD40, CD40L* and *BLYS* genes that participate of B-cell co-stimulation, for association with *Plasmodium vivax* malaria, characterized by a non-lethal disease but its prolonged and recurrent infection with deleterious effects on personal well-being, growth and on the economic performance at individual, family, community, and national levels. *P. vivax* is the most prevalent malaria species in Brazil it represents more than 80% of clinical cases reported annually from the Amazon region. The parasite-host coevolutionary process can be viewed as an arms race, in which adaptive genetic changes in one are eventually matched by alterations in the other, in this case, within the genetically diverse Amazonian populations**.** The sample included 97 patients and 103 controls. We extracted the DNA by using the extraction and purification commercial kit and identified the SNPs -1 C>T in the gene *CD40*, -726T>C in the gene *CD40L* and the -871C>T in the gene *BLyS* by the PCR-RFLP method. We analysed the genotypic, allelic frequencies, as well as of those individuals carrying each allele, by direct counting. We also compared the observed genotypic frequencies with the expected ones, according to the Hardy-Weinberg Equilibrium. The allelic, genotypic and allele carrier frequencies for these SNPs did not differ statistically between the patient and the control groups. Gene-gene interactions were no observed between CD40 and BLYS, and between CD40L and BLYS. Overall, the genes were balanced according to Hardy-Weinberg Equilibrium. The results of this study lead us to conclude that, although the CD40, CD40L and BLYS alleles analysed differ functionally, this variation does not alter the functionality of the molecules in a way that would interfere with the susceptibility of the disease.

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REDUCED ANTIBODY RESPONSES AGAINST *PLASMODIUM FALCIPARUM* VACCINE CANDIDATE ANTIGENS IN THE PRESENCE OF *TRICHURIS TRICHIURA*

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Helminths may affect immune responses to vaccines. In the target group for malaria vaccines helminth prevalence is high. Twenty Gabonese preschool-age children were vaccinated with GMZ2, a blood stage malaria

vaccine candidate. Humoral immune response against the vaccine antigens and parasitological status were assessed. Antibody response to GMZ2 was 3.4-fold (95% confidence interval: 1.6, 7.4) higher in *Trichuris trichiura* negative subjects compared to positive participants. Immunoglobulin subclass distribution was similar, whereas memory B-cell response tended to higher in *T. trichiura* negative individuals. Future malaria vaccine development programs need to account for worm-mediated hyporesponsiveness of immune reactions.

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IMPACT OF IPTC IN ANTIBODIES MSP-119 AND AMA-1 IN SENEGAL

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Malaria remains a major disease in many African countries, caused an estimated 243 million cases of clinical malaria and 863 thousand deaths globally 2008. The acquisition of immunity to clinical malaria is usually acquired the first five years of life depending on the intensity of malaria transmission. Nowadays, many strategies such as IPTic /SP are used for prevention in children. Intermittent Preventive Treatment for children (IPTc) against *Plasmodium falciparum* malaria is administered at defined intervals curative doses independently of the presence of parasites or symptoms. IPTc could however delay the acquisition of the antibodies which are managed against the malaria to this group of children. In this optics we want to understand the impact of this strategy in kinetics of specific antibodies against malaria on the acquisition of antibody in children living in zone of unstable transmission. This study measure the kinetics of antibodies MSP-119 and AMA-1 by ELISA, which are recombinantes proteins specifically managed against the membrane of *P. falciparum*. To evaluate the impact of IPTic /SP on antigenic variation in rural areas of three districts, all children aged 11mths-10years, in Senegal. Our results show that young children under 5 years are the ones who produce most antibodies and this production increases significantly with age (p=0, 0001). Production of AMA-1 antibody is more important (27, 09 %) than MSP-119 antibody (13, 69 %). Control zone produce more antibodies than intervention zone, the PSP is a factor which can modifies the production of antibody. Seroepidemiology can provide key information on malaria transmission for control programmes, when parasite rates are low.

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ELEVATED LEVELS OF CD4+CD45RA-CD62L+CD11A+ ARE ASSOCIATED WITH MALARIA SEVERITY IN CHILDREN FROM WESTERN KENYA

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High endothelial venules from secondary lymphoid organs display high levels of CD62L (L-selectin) ligands. In addition, murine T cells that undergo activation in secondary lymphoid organs up-regulate CD11a (LFA-1), an adhesion molecule that mediates interaction with activated endothelia and recruitment of activated T cells to inflamed regions. However, the role of CD11a and CD62L in the development of malaria severity remains largely unexplored. As such, we characterized CD4+ T-cell populations in parasitemic children (n=54; aged 12-36 months) presenting with varying severities of malarial anemia at Siaya District Hospital (SDH), in western Kenya. Complete hematological profiles were obtained with a Beckman Coulter Counter®, while Giemsa-stained slides were used to determine parasitemia. Participants were stratified based on hemoglobin (Hb) status as uncomplicated malaria (UM; Hb≥11.0 g/dL; n=12), mild malarial anemia (M/MA; Hb≥8.0<11.0 g/dL; n=22), and severe malarial anemia (SMA; Hb<6.0 g/dL; n=20). Venous blood was stained with anti-(CD3; CD4; CD45RA; CD11a and CD62L) antibodies. Cells were then acquired using four-color FACSCalibur™ flow cytometer. These results demonstrated that the proportion of (CD4+CD45RA-CD11a+) were comparable across the groups [median (IQR) UM, 98.16% (1.97); M/MA, 97.41% (2.71); SMA, 97.43% (2.69); *P*=0.422]. In addition, the proportions of (CD4+CD45RA-CD62L+) was comparable across the groups [median (IQR) UM, 72.52% (11.90); M/MA, 76.88% (12.80); SMA, 77.33% (11.90); *P*=0.229]. However, the co-expression of CD11a and CD62L (CD4+CD45RA-CD11a+CD62L+) differed significantly across the groups [median (IQR) UM, 67.98% (5.96); M/MA, 75.46% (15.07); SMA, 72.17% (9.89); *P*=0.025]. Further analysis showed that both the M/MA (*P*=0.012) and SMA (*P*=0.040) groups had elevated levels of circulating CD4+CD45RA-CD11a+CD62L+ relative to the UM group. These results suggest that CD4+ T cells co-expressing CD11a and CD62L are associated with malaria severity in this holoendemic transmission area, and may be important in the pathogenesis of SMA.

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VARIATION IN THE *HSPA1A* GENE LOCUS IS ASSOCIATED WITH SUSCEPTIBILITY TO SEVERE MALARIAL ANEMIA

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Heat shock protein (HSP) 70 is an important stress-inducible protein known to play a dual role as a molecular chaperone and immune modulator. Under normal physiological conditions, HSPs are constitutively expressed at low levels, but show dramatically increased expression during cellular stress and infection. Although polymorphic variation in the genes encoding HSP70 protein have been implicated in the pathogenesis of several diseases, their role in *Plasmodium falciparum* malaria has not been reported. In this context, we investigated the functional role of polymorphic variations in the promoter region of the *HSP70* gene [i.e., *HSPA1A* -217C/G (rs1043618), -5457A/C (rs2227955), and -5893G/A (rs34814308)] in conditioning malaria disease pathogenesis in children from a holoendemic *P. falciparum* transmission region of western Kenya. Parasitemic children (n=855; aged 3-36mos.) were stratified according to hemoglobin (Hb) levels into severe malaria anemia [(SMA), Hb<5.0 g/dL] and non-SMA (Hb≥5.0g/dL). Multivariate logistic regression analyses (controlling for covariates) demonstrated that the homozygous mutant (GG) at the -217 locus was associated with increased susceptibility to SMA [Odds ratio (OR), 2.736; 95% CI, 0.948-7.898; *P*=0.063], while the heterozygous (GA) at the -5893 locus was protective against SMA (OR, 0.538; 95% CI, 0.307-0.943; *P*=0.030). Haplotypic analyses revealed that carriage of the CAA (-217C/-5457A/-5893A) haplotype was associated with reduced risk of developing SMA (OR, 0.531; 95% CI, 0.298-0.947; *P*=0.032) and elevated levels of *HSPA1A* transcripts (*P*=0.009). Further examination of the relationship between malaria-associated inflammatory mediators and hsp70 regulated genes demonstrated that carriers of CAA haplotype had significantly lower production of IL-1β, IL-6 and TNF-α (*P*<0.05 for all). Taken together, these findings demonstrate that variation in the HSPA1A promoter is associated with susceptibility to SMA and production of inflammatory mediators known to affect the pathogenesis of SMA.

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REDUCED SYSTEMIC BICYCLO-PROSTAGLANDIN-E_2 AND CYCLOOXYGENASE-2 GENE EXPRESSION ARE ASSOCIATED WITH INEFFICIENT ERYTHROPOIESIS AND ENHANCED UPTAKE OF MONOCYTIC HEMOZOIN IN CHILDREN WITH SEVERE MALARIAL ANEMIA

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In holoendemic *Plasmodium falciparum* transmission areas, severe malaria primarily occurs in children <48 mos. and manifests as severe malarial anemia [SMA; hemoglobin (Hb) <5.0 g/dL]. Induction of high levels of prostaglandin-E_2 (PGE_2) through inducible cyclooxygenase-2 (COX-2) is an important host defense mechanism against invading pathogens. We have previously shown that COX-2-derived PGE_2 levels are reduced in children residing in hyperendemic transmission regions with cerebral malaria and in those with mixed sequelae of anemia and hyperparasitemia. Our *in vitro* studies further demonstrated that reduced PGE_2 was due to down-regulation of COX-2 gene products following phagocytosis of malarial pigment (hemozoin, *Pf*Hz). However, since COX-2-PGE_2 pathways and the impact of naturally acquired *Pf*Hz on erythropoietic responses have not been determined in children with SMA, plasma and urinary bicyclo-PGE_2/creatinine and leukocytic COX-2 transcripts were determined in parasitized children (<36 mos.) stratified into SMA (n=36) and non-SMA (Hb≥5.0 g/dL; n=38) groups. Children with SMA had significantly reduced plasma (P=0.001) and urinary (P<0.001) bicyclo-PGE_2/creatinine, and COX-2 transcripts (P=0.007). There was a significant positive correlation between Hb and both plasma (r=0.363, P=0.002) and urinary (r=0.500, P=0.001)] bicyclo-PGE_2/creatinine. Furthermore, decreased systemic bicyclo-PGE_2/creatinine was associated with inefficient erythropoiesis (i.e., reticulocyte production index; RPI<2.0, P=0.026). Additional analyses demonstrated that plasma (P=0.031) and urinary (P=0.070) bicyclo-PGE_2/creatinine and COX-2 transcripts (P=0.026) progressively declined with increasing concentrations of naturally acquired *Pf*Hz by monocytes. Results presented here support a model in which reduced COX-2-derived PGE_2, driven in part by naturally acquired *Pf*Hz by monocytes, promotes decreased erythropoietic responses in children with SMA.

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THE EFFECT OF CHANGING MALARIA TRANSMISSION ON THE ACQUISITION AND MAINTENANCE OF IMMUNITY TO MALARIA IN PREGNANT MALAWIAN WOMEN

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Malaria in pregnancy can have severe effects on both the mother and the foetus, including severe anaemia, low birth weight, congenital malaria, abortion, as well as maternal or infant mortality. These severe manifestations are often accompanied by the accumulation of *Plasmodium falciparum* (Pf)-infected erythrocytes (IE), pigmented monocytes and fibrin deposits in the placenta. Studies have identified VAR2CSA PfEMP1-expressing Pf that bind to chondroitin sulfate A in the placenta. In areas of high transmission, susceptibility to placental malaria decreases with increased gravidity, suggesting a protective role for antibodies (Abs) generated against placenta-binding Pf. The prevalence of malaria in pregnant women has decreased in some regions over the last decade with the increased use of insecticide-treated bed nets and intermittent preventative treatment during pregnancy. In this study, we investigate the impact of decreased malaria transmission on the acquisition of immunity to placental malaria (PM) and the maintenance of immunity to non-pregnancy malaria (NPM) among 500 pregnant women in Malawi over the period 1999 to 2006, when malaria prevalence decreased by approximately eighty per cent. To measure the acquisition of immunity to PM, we perform a number of assays to assess sera for protective Abs that bind to VAR2CSA PfEMP1-expressing Pf-IE, that prevent IE-placental adhesion and Abs that can opsonise IE for clearance by phagocytic cells. Maintenance of immunity against NPM during this period will be assessed by performing the same assays against CD36-binding PfEMP1-expressing Pf-IE, and by assaying for Abs against merozoite antigens.

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ANTI-PARASITE ANTIBODY RESPONSE ELICITED BY *PLASMODIUM FALCIPARUM* UNCOMPLICATED MALARIA

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Malaria results in the death of hundreds of children and pregnant women every week in tropical countries where the disease is endemic. Nevertheless, repeated *Plasmodium falciparum* malaria episodes do confer immunity against parasite infection, reducing the severity and morbidity attributed to the disease. However, such anti-malarial immunity gradually subsides and is not well understood. In a prospective cohort study carried out at Kasangati Health Centre in Wakiso district of Uganda, patients with uncomplicated *P. falciparum* malaria were enrolled, consented, treated with policy regimen (artemether-lumefantrine), demographics and clinical data obtained, all on day 0. Venous blood was collected on the same day of diagnosis and treatment (day 0) and at 42 days later (day 42), filter-paper dot blots were prepared and serum immunoglobulin antibodies against synthetic peptides representing *P. falciparum* candidate antigens were evaluated by ELISA. The studied peptides correspond to antigenic domains within i) glutamine rich protein (GLURP), ii) merozoite surface protein 3 (MSP3), and iii) histidine rich protein II (HRPII). Two hundred and fifty patients of 1 to 60 years of age were enrolled. Anti-*P. falciparum* peptide IgG levels assessed on basis of ELISA absorbance increased with age, especially anti-GLURP peptide IgG. Indeed, mean anti-GLURP IgG significantly increased from day 0 to day 42 but anti- MSP3 and anti-HRPII IgG did show similar increase. Although the sample size of HIV infected enrolled patients was small, we noticed that the mean parasite density of patients with CD4 lymphocyte counts less than 200 CD4/uL was higher than the corresponding values for HIV-negative patients and HIV-infected patients possessing higher than 200 CD4/uL.

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CONSEQUENCES OF MALARIA DURING PREGNANCY ON IMMUNOLOGICAL RESPONSES OF THE NEWBORN: A STUDY OF REGULATORY T CELLS

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Characterization of the factors linked to the highter susceptibility of the newborns to *Plasmodium falciparum* infection is a priority for the validation of any anti-malarial vaccine. Regulatory T cells (Treg) part of the natural pool of $CD25^+$ $CD4^+$ T cells play a major role in the maintenance of peripheral tolerance to self-antigens, and in the regulation of adaptive and innate immune responses against pathogens by controlling inflammatory responses and lymphocyte homeostasis. Treg are characterized by the phenotype $CD4^+$ $CD25^+$ $CD127^-$ $Foxp3^+$. Treg prevalence was observed to be higher in cord blood from a *P. falciparum* infected placenta, compared to uninfected placenta, with an effect on cellular immune responses, suggesting *in utero* stimulation on Treg by soluble *P. falciparum* antigens with altered pro-inflammatory responses. Our objective is to evaluate the impact of pregnancy associated malaria (PAM) and malaria during the first year of life on the profile of Treg at birth, and at 3, 6 and 12 month of age. Our hypothesis is that *in utero* contact with *P. falciparum* soluble antigens may modify the profile of Treg and we questioned the consequences of those changes on outcomes of infection during the first year of life. This study is part of the STOPPAM project a clinical and parasitological follow up of 1000 women during pregnancy and 200 infants during the first year of life in Comé, southwestern Benin. Immunophenotyping of Treg cells has been evaluated using flow cytometry in 158 infants in cord blood and again in peripheral blood at 3, 6 and 12 months of age from mothers with different malaria histories during pregnancy. Our results from a multivariate analysis show first, a significant increased of Treg frequency with infant age as described in newborn. Importantly, we observed a significant increased of Treg frequency at 3 month of age when the child was born of infected mothers at delivery. Our results suggest an impact of PAM on newborn cellular immune responses with an alteration in the control of pro-inflammatory responses necessary for the removal of parasites.

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CONSEQUENCES OF MALARIA DURING PREGNANCY ON NEONATAL ANTIGEN PRESENTING CELL ACTIVATION VIA TOLL-LIKE RECEPTORS AND *PLASMODIUM FALCIPARUM* ANTIGENS IN BENIN

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During pregnancy and early childhood, there is an increased susceptibility to malaria due to parasite-induced modulation of pro-inflammatory responses. The development of a protective immune response requires correct function of Toll-like receptors (TLR) expressed by antigen-presenting cells (APC). TLR activation induces cytokine production and expression of co-stimulatory molecules to trigger antigen presentation to T cells. In newborns, stimulation of APC via their TLR is involved in the progressive development of immune responses during the first months of life and our hypothesis is that pregnancy-associated *P. falciparum*-malaria may adversely affect this development. The study is part of the STOPPAM project conducted in Benin; a clinical and parasitological follow-up of 200 pregnant women and their children followed from birth to 12 months. To assess the impact of malaria on neonatal immunity peripheral whole blood of children (0, 3, 6 and 12 months) was stimulated either by TLR ligands (polyI:C, LPS, R848, CpGODN) that have distinct effects on APC subsets. Concentrations of pro- and anti-inflammatory cytokines were then evaluated in culture supernatants to investigate activation levels of APC. We performed the stimulations and cytokine assays on blood from 134 newborns from mothers with different malaria histories during pregnancy in order to evaluate the impact of an *in utero* contact with *P. falciparum* antigen on the newborn immune system. As a general trend, the responses of APC to TLR stimulation increased with age. According to a multivariate analysis, the mother's infection at delivery had a significant effect on infant immune responses: higher concentrations of IL6 in response to CpGODN and polyI.C at birth; of IL-10 in response to CpGODN and polyI.C at 3 and 12 month; of TNF-α in response to CpGODN, polyI.C and R848 at 6 months. Our findings suggest that mother's malaria infection at delivery altered the neonatal innate immune responses via TLR activation that may have consequences in the development of immune responses.

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PLASMODIUM FALCIPARUM CLEARANCE RATES IN RESPONSE TO ARTESUNATE IN MALIAN CHILDREN WITH MALARIA

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Artemisinin resistance, defined as a long half-life ($T_{1/2}$) of parasite clearance in response to artesunate, was recently described in patients with *Plasmodium falciparum* malaria in Cambodia and Thailand. $T_{1/2}$ values have not yet been reported from Africa, where artemisinin-based combination therapies were recently introduced. In Mali, we sought to establish a baseline parasite clearance rate in our study population and to investigate the contribution of acquired immunity to parasite clearance rates in response to artemisinins. In 2010 and 2011, we measured $T_{1/2}$ in 215 Malian children aged 0.5-15 years with uncomplicated *P. falciparum* malaria (10,000 – 100,000 parasites/µl). We provided directly-observed, weight-based doses of artesunate (0, 24 and 48 h) and amodiaquine (72, 96 and 120 h) orally, and counted the parasite density (/µl) in the blood every 6 hours until it was undetectable. From plots of $\log_e$-transformed parasite densities *vs.* time, we calculated $T_{1/2}$ and evaluated the effects of age, sex, ethnicity and red blood cell (RBC) polymorphisms on this parameter. The geometric mean $T_{1/2}$ was 1.93 hours (95% CI 1.85-2.01), significantly shorter than in western Cambodia (5.8 h). In a linear regression model that accounted for host factors, $T_{1/2}$ decreased by 4 minutes for every 1-year increase in age (r=-0.3117, p<0.0001). For 48 children, we quantified the proportion of parasitized RBCs recognized by autologous plasma IgG. The proportion of parasitized RBCs recognized by autologous plasma IgG ranged from 4%-76%. IgG responses increased significantly with age (r=0.3831, p=0.0072) and correlated inversely with $T_{1/2}$ (r=-0.475, p=0.0006). Our data indicate that acquired IgG responses to parasitized RBCs shorten $T_{1/2}$ and suggest that clearance of *P. falciparum* is in part achieved by IgG responses. These IgG responses may also contribute to $T_{1/2}$ values in areas like Southeast Asia, where age is an inadequate surrogate of immunity and no *in vitro* correlate of parasite-clearing immunity has been identified.

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KINETICS OF ANTIGEN-PRESENTING CELL POPULATIONS AFTER CONTROLLED HUMAN MALARIA INFECTION

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Antigen-presenting cells (APCs) are key players in the induction and regulation of immune responses via antigen-presentation, co-stimulation and production of cytokines and chemokines. In malaria, it remains elusive whether APCs, and particularly dendritic cells (DCs) are appropriately activated to subsequently carry out their function. In naturally exposed individuals living in endemic areas, blood DC activation seems to be impaired and *in vitro* studies suggest a weakened function of human monocyte-derived DC after *Plasmodium falciparum* (*Pf)* exposure. On the contrary, other *in vivo* and *in vitro* experiments show activation of DCs by *Plasmodium*. In most studies, the effect of the malaria parasite on DCs seems highly dose-dependent. We investigated the effects of low numbers of *Pf* on APCs in a first encounter *in vivo*, over the course of a controlled human malaria infection. Fifteen healthy Dutch malaria-naive volunteers were infected by intradermal injection of cryopreserved *Plasmodium falciparum* sporozoites (PfSPZ Challenge). Peripheral blood mononuclear cells (PBMC) were collected and cryopreserved at baseline, at several time points during liver-stage and blood-stage of the malaria infection and on days 35 and 140 after infection. We analyzed the cells by phenotypic staining followed by 9-colour flow cytometry. We will present the kinetics in composition of the blood-APC compartment (including BDCA-1, BDCA-2, BDCA-3 and CD16 DC subsets as well as classical and non-classical monocytes) and changes in activation, co-stimulatory and chemokine marker expression in the context of human liver- and blood-stage malaria infection.

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HLA-DR4 MOLECULES SUPPRESS PROTECTIVE ANTIBODY RESPONSES TO MALARIA PY17XNL BLOOD STAGE PARASITES

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Plasmodium falciparum is the most virulent and deadly malaria parasite that infects annually >200 million people and accounts for more than 650,000 deaths. HLA-DR4 expression in humans infected with *P. falciparum* has been associated with severe malaria and high mortality rate. Herein, we show that humanized mice expressing HLA-DR4 molecules and lacking mouse MHC class II molecules (AbbKO mutation) have impaired production of specific antibodies to normally non-lethal *P. yoelii* 17XNL blood stage parasites and succumb to infection. In contrast, F1 hybrid mice co-expressing HLA-DR4 and mouse MHC-II molecules as well as mice expressing HLA-DQ6, HLA-DQ8 or HLA-DR3 molecules on AbbKO background, were able to elicit antibodies and to self-cure the infection. As compared to BALB/c, C57BL/6, and the F1 hybrids, the HLA-DR4.AbbKO mice had an increased frequency of $CD4^+Foxp3^+$ regulatory T cells (Tregs) upon infection, and *in vivo* Treg depletion enabled them to elicit antibodies and self-cure the infection. These results demonstrated that HLA-DR4 expression associates with suppression of protective humoral responses to Py17XNL parasites. These results are consistent with clinical associations between HLA-DR4 and severe *falciparum* malaria.

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PROFESSIONAL ANTIGEN PRESENTING CELLS, NOT INFECTED HEPATOCYTES, INDUCE PROTECTIVE IMMUNE RESPONSES TO *PLASMODIUM FALCIPARUM* CSP IN *P. BERGHEI* TRANSGENIC MOUSE MODEL

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The role of Circumsporozoite Protein (CSP) in protection against malaria is controversial. While studies in rodent models indicated that CSP is not required for protection upon immunization with whole sporozoites -either irradiated or live under chloroquine cover- the role of *Plasmodium falciparum* CSP (PfCSP) upon vaccination of humans with whole parasites

is still uncertain. Also, the role of infected hepatocytes *vs* professional antigen-presenting cells in the presentation of PfCSP in an immunogenic form remains unclear. Here we show that mice injected intravenously with live *P. falciparum* sporozoites, which cannot infect mouse hepatocytes, elicited high titers of CSP-specific antibodies and were protected (>60%) against challenge with transgenic *P. berghei* sporozoites expressing PfCSP, but not against challenge with wild type *P. berghei* sporozoites. The results give evidence that PfCSP is a protective antigen, and highlight a critical role of professional antigen-presenting cells in stimulating protective immunity to PfCSP. This study supports efforts for using CSP subunit vaccines against human malaria.

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STUDY OF ANTIBODY MEDIATED CORRELATES OF PROTECTION AGAINST MALARIA IN THE MOUSE MODEL

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A near full length circumsporozoite protein (CSP) of *Plasmodium falciparum* (Pf) was expressed in *E. coli*, and purified to homogeneity. Pf CSP contains an N-terminal region, a central repeat (R) region and a C-terminal cysteine rich region. While several studies have shown the R-specific antibodies are the primary mediators of protection, the N- and C-terminal region-specific antibodies have also been implicated in protection. In the present study we dissected the antibody response to CSP to determine if the quantity and quality of antibodies against CSP could predict protection in a transgenic *P. berghei* parasite challenge model. The transgenic parasite expresses the *P. f* CSP gene in a rodent malaria (*P. berghei)* parasite, as reported previously. Mice vaccinated with CSP using a variety of adjuvants and delivery systems were challenged with the transgenic parasites. Protection was inferred if blood stage parasites were absent 15 days post challenge. Antibody correlates that were examined included ELISA titers against the full length, repeat and C-terminal region of CSP, antibody isotypes analyzed by luminex, avidity by thiocyanate method, IFA on fixed sporozoites and inhibition of sporozoite invasion into hepatocytes. Monoclonal antibodies are also being produced against CSP for passive transfer and competition ELISA. The data indicated that antibodies contribute to protection and certain correlates could distinguish protected from non-protected mice. These data have broader implications for down-selecting improved CSP based vaccine formulations for human trials.

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TRANSFORMING GROWTH FACTOR B-1 LEVELS CORRELATE WITH PLATELET COUNT, RANTES LEVELS AND SEVERITY OF DISEASE IN *PLASMODIUM FALCIPARUM* MALARIA

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A balance between pro- and anti-inflammatory cytokines appears to be necessary to defend against and survive malaria. Transforming growth factor beta-1 (TGF- β1), a cytokine that regulates inflammation, has inflammatory properties at low levels and anti-inflammatory properties at higher concentrations. Platelets are a major source of TGF- β1, and platelet counts are reduced in severe malaria. However, the relationships between TGF- β levels and disease severity, platelet count and levels of other cytokines and chemokines are not well described. To assess these relationships, serum levels of TGF- β1 were assessed by ELISA, and IL-1β, IL-6, IL-10, IFN-γ, TNF- α, MIP-1α, MIP-1β, and RANTES by cytometric bead assay (CBA), in Ugandan children with cerebral malaria (CM, n=75), uncomplicated malaria (UM, n=67) or healthy community children (CC, n=62). TGF-β1 levels decreased with increasing severity of disease (median levels in pg/ml in CC, 44.0 (16.8-82.5), UM, 25.7 (0.8-70.6), CM, 14.1 (1.9-39.3), *P* for trend<0.0001). In children with CM or UM, TGF- β1 levels on admission correlated positively with platelet count (CM, Spearman's rho=0.61, P<0.0001, UM, rho= 0.38, P=0.0015), and RANTES levels (CM, rho = 0.56, P<0.0001; UM, rho= 0.48, P<0.0001). In children with CM, TGF- β1 levels on admission correlated negatively with IFN-γ (rho=-0.39, P=0.001). TGF- β1 levels were not associated with death or with adverse neurologic or cognitive outcomes. The study data suggest that reduced levels of TGF- β1 and RANTES in severe malaria may be due to the low platelet count seen in severe malaria. In turn, reduced TGF- β1-mediated regulation of pro-inflammatory cytokines may lead to increased levels of IFN-γ and other pro-inflammatory cytokines, and thus lead to more severe disease. Further research is required to elucidate the temporal relationships between these factors.

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APOPTOSIS OF NON-PARASITIZED RED BLOOD CELLS IN *PLASMODIUM YOELII* MALARIA

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Recently, through the study of erythrocytic apoptosis during *Plasmodium yoelii* infection, we observed a rise in the levels of non-parasitized red blood cells (nRBC) apoptosis that could be associated with the development of severe malaria anaemia, as premature elimination of nRBC is a relevant mechanism leading to this malaria complication. In the present study, we attempt to investigate the participation of nRBC apoptosis in malaria anaemia as well as the influence of parasite load and immune response on this cell death process. Balb/c mice were intraperitonially infected with *P. yoelii* 17XL and, then, nRBC apoptosis as well as number of peripheral RBC, parasitemia and plasmatic levels of cytokines, nitric oxide and anti-RBC antibodies were evaluated at early and late stages of infection. Apoptosis of nRBC was increased only at the late stage of infection and it was related to parasite load, but not to the intensity of the immune response. In spite of increased percents of nRBC apoptosis observed when the anaemia degree was accentuated, this increase was not associated to the reduction of peripheral RBC counts. We conclude that nRBC apoptosis in malaria can be induced in response to high parasite load and that this apoptotic process does not significantly contribute to the anaemia observed in the malaria model studied herein. Further studies on malaria models in which acute anaemia develop under low parasite burden could help to identify the potential pathogenic role of nRBC apoptosis.

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DEVELOPING NOVEL SEROLOGIC ASSAYS OF MALARIA EXPOSURE AND PROTECTION

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Current methods for assessing *Plasmodium falciparum* (Pf) transmission intensity are labor intensive and inaccurate, limiting our ability to assess effects of control interventions. In particular, there are no standard biomarkers to assess exposure or immunity to malaria parasites. Antibody responses to Pf develop as a function of the number and timing of Pf exposures. Kinetics of antibody responses to specific Pf antigens have only been evaluated for a small subset of antigens, but have been shown to differ. Specific antibody responses are known to provide protection from malaria, but protective responses are poorly defined. We propose that assessment of antibody responses to appropriately selected Pf antigens will allow estimation of recent and cumulative exposure to Pf and estimation of protection from malaria. Previously, we probed a microarray containing ~23% of the Pf proteome with plasma from 220 subjects in Mali and identified serologic responses to 49 Pf proteins associated with protection. Here, we analyzed these microarray data to identify novel markers of prior exposure and additional biomarkers of protection to Pf. 47 antigens were identified as candidate markers for cumulative exposure by selecting responses with the best linear fit of increased antibody intensity with increasing age in children 2-10 years. 78 candidates for recent exposure were identified as responses with either the most significant difference in reactivity between the beginning and end of the malaria transmission season or the best ability to predict elapsed time since last parasitemia, assuming an exponential decay of antibodies. 49 additional candidates for protection were identified by variable importance indices in random forest analysis. Based on these analyses, an array with 185 unique proteins will be tested with longitudinal samples from Ugandan children in a wide variety of transmission settings in an attempt to translate estimates of Pf exposure and protection at an individual level to estimates of transmission intensity and immunity at a population level.

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EVALUATION OF ANTIBODY TITERS TO *PLASMODIUM FALCIPARUM* AND *P. VIVAX* MEROZOITES SURFACE PROTEIN-1 (MSP-1) IN CAMBODIAN ADULTS DEVELOPING UNCOMPLICATED MALARIA DURING AN OBSERVATIONAL COHORT STUDY

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Merozoite surface protein-1 (MSP1), a protein involved in erythrocyte invasion, is found on the surface of the malaria blood stage merozoite. In addition to being a marker of past malaria exposure, there is evidence for a protective role of MSP1 antibodies against malaria infection. As part of an active, observational cohort with a nested treatment study evaluating the efficacy of dihydroartemisinin-piperaquine (DP) for uncomplicated malaria, we conducted a sero-epidemiological analysis for the prevalence of antibodies to both *Plasmodium falciparum* and *P. vivax* MSP1 in service members of the Royal Cambodian Armed Forces between October 2010 and February 2011, typically considered a period of moderate to low seasonal transmission in Cambodia. Of 256 healthy adult volunteers screened, 222 were enrolled with 89 developing primary uncomplicated malaria infection with 32 volunteers having a recurrence after DP treatment. The majority of primary infections (75%) were *P. vivax* with the remaining 25% being *P. falciparum*. Using a titer of 450 units as a cutoff based on non-immune Thai adult blood donors, 76% and 56% of volunteers had titers above cutoff to *P. vivax* and *P. falciparum* respectively. Titers to *Pv* MSP1 at baseline ranged from 65-965,671 units with a geometric mean titer (GMT) of 2207 units (95% CI 1746-2789); the range of *Pf* MSP1 titers at baseline was 16-669,774 units with a GMT of 904 (95% CI 703-1162). For volunteers who were treated for malaria, *Pf* MSP1 titers at time of infection did not change, but titers for *Pv* MSP1 doubled, returning to baseline at discharge. Uninfected volunteers had a lower GMT at discharge than baseline, possibly reflecting less exposure at the end of the transmission season. The individual and grouped serological data will also be analyzed in relationship of antibody titer at baseline infecting genotype and species at time of infection as well as to recurrences after curative treatment with DP.

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ANTENATAL MALARIA INFECTIONS ARE ASSOCIATED WITH IMPAIRED HIB AND DIPHTHERIA VACCINE IMMUNE RESPONSES IN KENYAN CHILDREN

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Antenatal maternal parasitic infections prime the fetal immune response and induce an immunomodulatory phenotype at birth that may affect subsequent immune responses to commonly administered childhood vaccines. Here we examined the effect of malaria, schistosomiasis and filariasis in pregnant women on the responses to Haemophilus influenzae type B (Hib) and diphtheria (DT) vaccination in their offspring. 241 infants were followed every 6 months from birth to 3 years and IgG antibody levels to polyribitol-ribose phosphate (PRP Hib) and diphtheria toxoid (DT) were measured by ELISA. Linear mixed-effects models were used to characterize PRP and DT responses over 6 to 36 months of age. Offspring of malaria-infected women tended to have lower PRP levels compared to offspring of women without known malaria infection during pregnancy although none of these comparisons were significant. By contrast, classification of children based on their mother's malaria infection status and on cord blood (CB) recall responses to malaria blood-stage antigens as putative tolerant (mothers with malaria [Pf+] but lacking detectable Th1 and Th2-type recall responses to malaria-blood stage antigens, N=12), sensitized (Pf+ women and detectable Th1 and Th2-type recall responses in CB to malaria, N=153) or unexposed (mothers Pf- and lack of CB lymphocyte responses, N=75) had a significant impact on PRP- and DT-specific IgG levels. Putatively tolerant children had lower DT- and PRP-specific IgG levels at 24, 30 and 36 months of age to PRP (P=0.37, <0.04 and <0.005) and DT (P=<0.02, 0.006 and <0.04) compared to unexposed children. Initial analysis indicates maternal schistosomiasis and/or filarial infection during pregnancy may have less impact on PRP- and DT-specific IgG levels than that observed with maternal malaria infections. Thus, malaria during pregnancy may impair childhood vaccine efficacy and highlight the importance of programs to eradicate parasitic infections in pregnant women.

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FIRST EVIDENCE OF PYRETHROID RESISTANCE IN AN *ANOPHELES FUNESTUS* POPULATION FROM SENEGAL

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Anopheles funestus is one of the major malaria vectors in tropical Africa notably in Senegal. The highly anthropophilic and endophilic behaviours of this mosquito make it a good target for vector control operations through the use of insecticide treated nets, long-lasting insecticide nets and indoor residual spraying. However, little is known about the resistance status to insecticides of field populations of this vector in Senegal and the potential underlying resistance mechanisms. To fill this gap in our knowledge, we assessed the susceptibility status of *An. funestus* populations from Gankette Balla, located in the Senegal River Basin. WHO bioassays indicated that An. funestus in Gankette is resistant to lambda-cyhalothrin 0.05% (74.32% mortality / n = 222). Suspected resistance was observed to deltamethrin 0.05% (87.72% mortality / n = 114), permethrin 0.75% (91.37% mortality / n = 139), DDT 4% (93.20% mortality / n = 147), bendiocarb 0.1% (94.27% mortality / n = 157) and dieldrin 4% (96.41% mortality / n = 306). However this population is fully susceptible to malathion 5 % (100% mortality / n = 50) and fenitrothion 1% (100% mortality / n = 55). Sequencing of the fragments of Voltage-Gated Sodium Channel did not detect the L1014F mutation. A microarray analysis indicated that the lambda-cyhalothrin resistance in Gankette is conferred by metabolic resistance mechanism under the control of P450 genes. This study represents the first report of pyrethroid resistance in An. funestus from Senegal. These findings should be taken into account by malaria control programs and further studies are needed to establish the geographic distribution of this resistance across Senegal.

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INCREASED LEVELS OF PYRETHROID RESISTANCE IN THE PRIMARY MALARIA VECTOR *ANOPHELES ARABIENSIS* IN RURAL LOWER MOSHI, NORTHEASTERN TANZANIA

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The major foci of pyrethroid resistance in 1990-2010 were in West and Central African populations of *Anopheles gambiae s.s.* In East Africa pyrethroid resistance has been recorded in relatively few locations and at low frequencies. Three cross-sectional surveys of *Anopheles arabiensis* were conducted to determine levels of resistance to organochlorines, organophosphates, carbamates and pyrethroids in Lower Moshi. Wild mosquitoes were hand-collected from cowsheds between April-June in 2004, 2009 and 2011 in several villages. Susceptibility tests were conducted using standard WHO diagnostic dosages and kits. Knockdown was recorded after 5, 10, 15, 20, 30, 40, 50, 60 minutes and mortality 24 h post-exposure was recorded. The first survey in 2004 of *An. arabiensis* indicated low levels of resistance to permethrin (84% mortality) and deltamethrin (87% mortality). *An. arabiensis* were fully susceptible to DDT (organochlorine), fenitrothion and malathion (organophosphates), and propoxur (carbamate).The 2009 survey data showed a slight increase in the frequency of resistance for lambdacyhalothrin (mortality <80% in 3 villages), and permethrin (67% mortality in one the villages). The mean mortality for all villages tested after exposure to deltamethrin was 92%. The recent 2011 survey revealed high levels of resistance to lambdacyhalothrin in all villages (mortality <60%) and deltamethrin in Msitu wa tembo (31% mortality). Resistance to permethrin remained moderate in most villages and similar to 2009. *An. arabiensis* were still fully susceptible to DDT, carbamates and organophosphates. These results clearly demonstrate the presence of pyrethroid resistance in *An. arabiensis* in Lower Moshi. The lack of DDT resistance coupled with previous studies showing very low frequency *kdr* suggests that enzyme-based mechanisms are responsible for resistance in *An. arabiensis*. Tanzania has recently scaled-up vector control programmes with universal coverage of pyrethroid LLINs and IRS in several regions in 2011. Regional monitoring of resistance should continue and provide an early warning so that alternative insecticides can be considered if resistance levels become operationally significant

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EVIDENCE OF HIGHLY FOCAL PATTERNS OF PYRETHROID RESISTANCE IN *AEDES AEGYPTI* IN IQUITOS, PERU

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Dengue fever is one of the most rapidly growing public health problems worldwide, with an estimated 50 million infections annually and 2.5 billion people living in areas at risk of infection. In the absence of a vaccine, control of dengue vector mosquitoes (principally *Aedes aegypti*) is the only available preventive measure. The city of Iquitos, located in the Amazon region of north-eastern Peru, is a highly dengue endemic city, with an estimated 60% of the population having been infected with at least one of the four dengue virus serotypes. Vector control activities rely heavily on the use of chemical insecticides to attack both larval and adult mosquito stages. The intensity of vector control responses to seasonal peaks in dengue transmission has increased in recent years, and the pyrethroid alphacypermethrin has been particularly heavily deployed as an adulticide during recent outbreaks. Although insecticide resistance was not previously reported in Iquitos, this intensive pyrethroid use appears to have increased selection pressure on local *Ae. aegypti* populations, as recent bioassay data indicate that pyrethroid resistance, particularly to lambdacyhalothrin and deltamethrin, is increasing within the city, but is limited to certain foci. Bioassay data also show widespread resistance to DDT, suggesting that cross-resistance patterns between DDT and pyrethroids may also be focal. Resistance to carbamates and organophosphates has not yet been detected. Data will be presented in terms of geographical and temporal variation, resistance intensity, and intensity of insecticide-based control activities over 2 dengue transmission seasons.

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NOVEL INSECTICIDE BIOASSAY BASED ON SUGAR FEEDING BY ADULT *AEDES AEGYPTI* (L.) *(DIPTERA: CULICIDAE)*

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Population monitoring to detect insecticide resistance in mosquitoes is an essential component of integrated disease management programs. We developed a bioassay method for assessing insecticide susceptibility based on the feeding activity of mosquitoes on plant sugars. The prototype sugar-insecticide feeding bioassay system was composed of inexpensive,

disposable components, contained minimal volumes of insecticide, and was compact and highly transportable. Individual mosquitoes were assayed in our feeding module consisting of a transparent plastic cup (30 mL) with a snap-on lid. The cup contained a feeding platform fitted with a receptacle holding a 10% sucrose-permethrin solution. Trypan blue dye was added to the sucrose-permethrin solution to create a visual marker in the mosquito's abdomen for ingested sucrose solution. Blue fecal spots deposited by a mosquito onto the feeding platform provided further evidence of solution ingestion. The proximity of the mosquito to the solution facilitated rapid ingestion. In time-course experiments, 100% of mosquitoes fed on the solution within 2 h of exposure. With the sugar-insecticide feeding bioassay, the permethrin susceptibility of *Aedes aegypti* females from two field-collected strains was characterized by probit analysis of dosage-response data. Further optimization of the device is possible to produce a concise, easy-to-mass produce and easy-to-read assay to measure insecticide susceptibility for mosquito adulticides.

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INSECTICIDE RESISTANCE STATUS OF THE ASIAN TIGER MOSQUITO IN THE UNITED STATES

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Aedes albopictus, the Asian tiger mosquito (ATM), the principal vector of chikungunya, is an introduced invasive species in the US responsible for a significant proportion of service requests to local mosquito control programs. ATM was first detected in the US in 1985 but is now one of the most common pest mosquitoes responsible for many service calls that result in the application of insecticides. However, to date very limited information is available on the insecticide resistance status of US ATM populations. Because of the possible impact of insecticide resistance on present and future ATM control operations we implemented the current studies. Specifically, we focused on the insecticide resistance status of ATM populations from New Jersey, Pennsylvania and Florida. Overall we tested nine populations (5 from NJ and 2 each from PA and FL). We implemented larval and adult bioassays following WHO standard protocols. We chose a range of insecticides representing classes or type of insecticides with different modes of action currently or historically used in the US for mosquito control (organochlorines, organophosphates, pyrethroids, carbamates, insect growth regulators and bioinsecticides). Larval bioassays revealed overall complete susceptibility to most insecticides but we did find some populations with reduced susceptibility to a carbamate. Similarly, most adults tested with WHO tube tests were fully susceptible to the majority of insecticide classes but surprisingly we found evidence of high levels of resistance concentrated in a few populations. To investigate the possible mechanisms involved in resistance such as metabolic-based resistance (Oxidases, Glutathione S-Transferases, and Esterases) and target-site resistance (kdr and ace mutations) we developed both biochemical and DNA based assays. In light of the results we will discuss the efficacy of different insecticide classes used for ATM control, and the resistance, or cross resistance patterns in US ATM that may threaten future control operations.

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ELECTRONIC DATA FOR INDOOR RESIDUAL SPRAYING (IRS): A PILOT TO CAPTURE, MAP AND MONITOR SPRAY ACTIVITIES IN ZAMBIA

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Indoor residual spraying (IRS) along with long-lasting insecticide treated nets (LLIN) form the mainstay of vector and subsequently malaria control throughout sub-Saharan Africa. Zambia has invested heavily in IRS over the past decade and now boasts 80% target coverage levels in urban/peri-urban settings contributing to a significant reduction in national malaria parasitemia from 22 % in 2006 to 16% in 2010. Historically, spray data is collected on paper, which is then manually aggregated and entered into a spreadsheet for reporting. Multiple spreadsheets are then manually combined to produce the final dataset. This system is labour intensive, prone to errors and limited in scope. To address this issue, an electronic data capture solution (mSPRAY) was developed and piloted in Chibombo district, Zambia, for rapid collection and dissemination of IRS data. IRS operators were individually equipped with a personal digital assistant (PDA) pre-loaded with the mSPRAY software that guides them through collection of all data elements including GPS coordinates for every structure, spray application details, LLIN usage and previous spray history. Validation rules built into the software ensured that only valid data was entered. Supervisors were able to review these data at any stage to increase accuracy. Periodically, datasets were exported for timely reporting to the district / provincial / central level(s). During the season, mSPRAY was able to provide regular feedback on overall performance. As a result it was able to identify a major shortfall in reaching total target structure coverage. Based on this data, operational changes were made to address this issue and coverage was dramatically improved. mSPRAY also identified areas missed during spraying that were originally targeted, again allowing spray teams to revisit these overlooked areas. In short, mSPRAY offers a robust and expanded data collection method allowing fine spatial mapping of spray activities to ensure that IRS applications are as effective and efficient as possible.

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EVALUATION OF LONG-LASTING ALPHA-CYPERMETHRIN IMPREGNATED NETS IN ATTRACTIVE LETHAL OVITRAPS (ALOT) AGAINST *AEDES AEGYPTI* FOR DENGUE CONTROL IN IQUITOS, PERU

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Dengue is the most important mosquito-borne viral disease in the world, and *Aedes aegypti* is the primary vector in the majority of affected tropical countries. Without an effective vaccine, chemical control of the adult vector is an important weapon in reducing disease impact. Our study was carried out in Iquitos, Peru, an urban city located in the Amazon basin, where dengue epidemics have been reported since 1990, with the largest in terms of morbidity and mortality occurring between 2011-2012. In June 2011, we initiated a large efficacy trial of a novel Attractive Lethal Ovitrap (ALOT) to reduce dengue and its mosquito vector. The ovitraps had 3 components: 1) a black and red colored outer structure that visually attracted mosquitoes; 2) an alpha cypermethrin 0.55% impregnated

long-lasting net that killed adults when they rested inside the trap; and 3) a packet containing a larvicide (spinosad) with lyophilized bacteria (attractant) applied to water in the base of the trap. We evaluated the efficacy of the net component (DuraNet™) of the ALOT traps between June 2011 and April 2012. We randomly selected twenty nets from approximately 7,000 traps deployed in approximately 2,800 houses and evaluated them in duplicate under lab conditions using two strains of *Ae. aegypti*: New Orleans and Iquitos. Nets without insecticide were used as controls. Mortality (24 hr %) ranged from 72-100% in the Iquitos field-derived strain and 99-100% in the New Orleans susceptible control strain. The results indicate that the net component of ALOT traps is maintained over an 8-month period under field conditions.

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MULTICENTRE STUDIES OF INSECTICIDE-TREATED DURABLE LINING IN AFRICA AND SOUTHEAST ASIA: ENTOMOLOGICAL EFFICACY AND HOUSEHOLD ACCEPTABILITY DURING ONE YEAR OF FIELD USE

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Indoor residual spraying (IRS) is a primary method of malaria vector control but its potential impact is constrained by several inherent limitations: spraying must be repeated when insecticide residues decay, householders can tire of the annual imposition and campaign costs are recurrent. Durable Lining (DL) is a deltamethrin-impregnated polyethylene sheeting material that can be used to cover walls and ceilings of domestic habitations that would normally be sprayed with insecticide. It can be considered a form of long-lasting IRS in which insecticide is released gradually from an aesthetically attractive wall covering. The operational success of DL will be contingent on attaining a high level of user acceptability as households need to maintain correctly installed materials on their walls for a number of years. A one year multicentre trial was conducted in 480 households from seven malaria endemic areas (Angola, Equatorial Guinea, Ghana, Mali, Nigeria, South Africa and Vietnam) representing the largest field evaluation of DL to date. At each site the durability, bioefficacy and household acceptability of DL was assessed compared to conventional IRS and other long-lasting insecticide-treated products. Over the year, the DL demonstrated little to no decline in bioefficacy, which was supported by a small loss of insecticide content. The majority of participants reported reductions in mosquito density (93%) and biting (82%), but no adverse changes to their indoor environment (83%). The DL was well received, more so in rural than in urban houses, because of its perceived efficacy and aesthetic value. When offered a choice of vector control product at the end of trial, DL always emerged as the most popular intervention regardless of the earlier household allocation. These results suggest that DL could overcome many of the user constraints associated with spray campaigns and has the potential to become a viable, long-lasting alternative to IRS in malaria endemic areas.

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SMALL-MOLECULE INHIBITION OF 'KIDNEY' FUNCTION IN MOSQUITOES

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About half of the world's population is at risk of contracting malaria, which kills 1 million individuals each year. Increased resistance in the malaria vector mosquito *Anopheles gambiae* is threatening the use of common insecticides such as pyrethroids. The development of novel insecticides with new modes of action is therefore essential for controlling mosquito populations and limiting malaria transmission. Malpighian tubules, the kidneys of mosquitoes, are essential for the survival of mosquitoes after they consume a blood meal, because they mediate the excretion of excess salts and water that are ingested. Our previous physiological studies of isolated Malpighian tubules have shown that barium-sensitive potassium channels are critical to the excretion of salt and water by this epithelium. Recently, we have cloned a barium-sensitive, inward rectifier potassium (Kir) from the Malpighian tubules of the yellow fever vector *Aedes aegypti* (AeKir1). We hypothesize that chemical disruption of AeKir1 will inhibit Malpighian tubule function and make mosquitoes more susceptible to the physiological stresses associated with blood feeding. Here, we report the discovery of a small-molecule antagonist termed VUXXX that inhibits heterologously expressed AeKir1 with a 50% inhibitory concentration of 10 μM, providing a tool compound for exploring the physiology and viability of AeKir1 as an insecticide target. Consistent with AeKir1 being essential for urine formation, VUXXX at a concentration of 5-10 μM inhibits fluid secretion in isolated Malpighian tubule assays. Medicinal chemistry is being used in an effort to increase the potency and selectivity of VUXXX for AeKir1 over mammalian Kir channels. In an effort to identify other inhibitors of AeKir1, we have developed and implemented a fluorescent-based high-throughput screening (HTS) assay to support a drug discovery campaign for AeKir1. This study is the first to target the mosquito renal system for the development of novel insecticides. Funding is provided by a grant from the Foundation for the NIH, VCTR program.

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PREVENTING THE SPREAD OF THE VIRUS VECTOR *AEDES ALBOPICTUS* TO THE AUSTRALIAN MAINLAND: A CAMPAIGN OF SUPPRESSION IN THE TORRES STRAIT ISLANDS

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Dengue is the leading arboviral health issue in Australia. There are hundreds of imported cases annually, and local transmission, mediated by *Aedes aegypti*, has resulted in multiple outbreaks in northeastern Queensland and the Torres Strait Islands (36 outbreaks and 2,365 cases since 2000). The region's Dengue Action Response Team (DART) is generally successful at constraining outbreaks and eliminating the virus. This is achieved through active case management and highly targeted vector control measures. Australia now faces a further threat from another dengue vector; *Ae. albopictus*. This is the world's most invasive mosquito. Its presence would dramatically complicate the epidemiology and control of dengue transmission in the region. Its climatic tolerances also predict that it would establish over a far greater area than *Ae. aegypti*. It represents a considerable public health risk and a public nuisance for Australia. It established on the Torres Strait Islands in 2005 but is yet to reach the mainland despite considerable traffic between these areas. This is largely because the DART conducts a campaign of vector suppression that focuses on the hubs of the major transport routes to the mainland.

Ae. albopictus is adapted to a more sylvan habitat than *Ae. aegypti*. Its management has therefore necessitated the careful development and adaptation of vector control tools and monitoring methods by the DART and the construction of an evidence base that demonstrates their utility. The campaign has been successful at reducing the *Ae. albopictus* population on the target islands to extremely low levels. In contrast, neighbouring untreated islands have experienced dramatically increased numbers. On the targeted islands, *Ae. aegypti* remains the commoner of the two species. Elsewhere it has been completely displaced by *Ae. albopictus*. This is the result of the differential susceptibility of these species to a program specifically targeted against Ae. albopictus. To date, the activities of the DART have been successful at protecting Australia from a mainland incursion. The sustainability of the approach is, however, unclear. A number of other tools for both vector control and disease mitigation are being explored.

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POTENTIAL FOR PYRIPROXYFEN TO STERILIZE *ANOPHELES ARABIENSIS* MOSQUITOES

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Achieving malaria elimination will require new ways to control insecticide resistant and outdoor biting/resting mosquitoes. The most abundant malaria vector in much of East Africa is *Anopheles* arabiensis which shows exophilic and exophagic behaviour and has developed pyrethroid resistance in some areas. The aim of this study was to determine whether Pyriproxyfen (PPF), a juvenile hormone regulator, could be used to sterilise those mosquitoes not successfully targeted with current control methods such as insecticide treated bed nets and indoor residual spraying. The bottle assay was used to expose mosquitoes to PPF at a rate of 0.003g AI/m2, with control mosquitoes subjected to the same conditions without the PPF. Differing levels of sterilisation were observed varying from none, to complete, depending on the time of blood feeding in relation to PPF exposure. Mosquitoes with fully developed eggs at the time of exposure, or fed post exposure showed little reduction in fecundity and fertility. Mosquitoes fed 1 day prior to, or during exposure, showed near complete reductions in fecundity and of the small number of eggs laid, none hatched into larvae. These results therefore open up new avenues for control measures for example in combining PPF into pyrethroid treated bed nets as a method of controlling resistant mosquitoes, or as a lure and sterilise technique for outdoor biting mosquitoes.

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EXPANDING THE FOOTPRINT OF *AEDES ALBOPICTUS* IN BELIZE, CENTRAL AMERICA

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The Asian tiger mosquito, *Aedes albopictus* (Skuse), is an aggressive daytime-biting mosquito closely associated with human activity and a vector of several viral diseases including dengue, chikungunya and yellow fever. Native to Southeast Asia, its status as an invasive species is well known. Against the backdrop of increased reporting of dengue fever cases, the first human-landing report of adult *Ae. albopictus* in Belize occurred in 2009 at Benque Viejo del Carmen, a city along the Guatemalan border in the Cayo District. We report here the first record of *Ae. albopictus* from the northern Orange Walk (OW) District, Belize and the insecticide resistance status of this population to malathion (Fyfanon®), the standard Belize MoH adult dengue vector control intervention.

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TOWARDS A PUSH-PULL STRATEGY FOR MALARIA VECTOR CONTROL: OUTDOOR MOSQUITO TRAP DYNAMICS IN NORTHERN BELIZE, CENTRAL AMERICA

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Current vector control tools are fast becoming inadequate to control malaria for a number of reasons, including insecticide resistance. With the intent to eliminate malaria, novel approaches are required. Our team is evaluating the use of spatial repellents and outdoor mosquito traps in a combined push-pull strategy to reduce the probability of human-vector contact. This study reports on the first of a series of experiments in Belize, Central America intended to develop and evaluate this integrated approach for malaria prevention. Two commercially available outdoor mosquito traps, the CDC miniature light trap and BioGents-Sentinel™ mosquito trap, were evaluated in a series of all-night experimental hut studies to compare trap densities of natural *Anopheles spp.* mosquitoes. Results indicate that CDC traps captured greater mosquito densities per hour than BG-Sentinel™ traps for three prevalent local malaria vectors: *An. vestitipennis*, *An. albimanus* and *An. punctimacula*. With the exception of *An. punctimacula,* mosquito entry patterns into experimental huts (i.e. peak entry time and overall numbers) were similar regardless of which trap was deployed. In a subsequent study, CDC traps captured an average of 80.6 *Anopheles* mosquitoes per night, but there was no significant impact on the number of *Anopheles* mosquitoes entering the huts compared to no-treatment controls. These findings suggest that while outdoor traps readily remove vectors from the peridomestic area, the impact on overall numbers of host-seeking mosquitoes was negligible. Moreover, those anophelines not captured were not deterred from entering huts occupied by human hosts. Future studies will quantify the effects of integrating indoor spatial repellent treatments on mosquito entry and on outdoor trap dynamics to define potential increased efficacy of an integrated push-pull approach.

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DEVELOPMENTAL PLASTICITY OF CONTAINER MOSQUITOES IN RESPONSE TO VARIABLE ENVIRONMENTAL CONDITIONS AND DENSITY

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Few studies have sought to determine relative importance of multiple environmental factors on developmental life-history traits of mosquitoes, despite the demonstrated effects of many single factors on individual variation. Understanding the drivers of development is of critical epidemiological importance in mosquito vectors in order to facilitate accurate prediction of populations in response to rapid, global climate change. We analyze the relative importance of multiple environmental conditions on the phenotype of development rate, assess possible interactions between environmental factors, and provide a measure of the phenotypic plasticity across gradients of environmental conditions in the yellow fever mosquito, *Aedes aegypti.* A meta-analysis of the empirical estimates of developmental studies over 100 years of research on *Ae. aegypti* allows us to search for broad phenotypic patterns over time and space. The meta-analysis reveals significant heterogeneity in developmental timing between studies. The environmental factor of temperature significantly explains this bulk of this heterogeneity in the literature. The meta-analysis is limited by literature bias because most studies have focused solely on temperature, and conduct experiments under limited ranges of instraspecific rearing density or food availability. As a result, other environmental factors known to potentially

influence development rate could not be detected. To address this, we experimentally estimate the norms of reaction in development rate of *Ae. aegypti* in controlled environmental chambers over wide gradients of multiple environmental conditions including temperature, food availability, and intraspecific rearing density. Results demonstrate that *Ae. aegypti* development rates are highly plastic across varying environmental conditions spanning poor to high resource quality. In contrast to the results of the meta-analysis, this developmental rate variation cannot be explained by temperature alone. Rather, the quality of the environment influences rate of development and is determined by multiple factors and significant, complex interactions between these factors.

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JAPANESE ENCEPHALITIS VECTOR MODEL DEVELOPMENT: AN AFHSC-GEIS NETWORK EFFORT

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Established in 1997 by Presidential Directive NSTC-7, the Department of Defense (DoD) Global Emerging Infections Surveillance and Response System (GEIS) was incorporated as a Division of the Armed Forces Health Surveillance Center (AFHSC) in 2008. The AFHSC-GEIS partner network includes five core DoD overseas laboratories and over 30 other partner institutions within the US and abroad. GEIS partners work in conjunction with host nations to conduct global disease surveillance that supports US Force Health Protection as well as local health interests. AFHSC-GEIS strives to maintain a centrally coordinated network in which their partners not only report to GEIS, but collaborate with one another to create stronger products that enhance DoD and host nation health systems for improving disease risk and threat reduction strategies. This has been accomplished in part by sustaining central laboratories that supply partners with reagents, laboratory and technical support, as well as by instituting internationally harmonized efforts in the fields of influenza and malaria surveillance. This collaboration has also been achieved in the development of disease risk and prediction models. One of the most salient examples is a distribution model for the primary Japanese encephalitis (JE) mosquito vector, *Culex tritaeniorhynchus,* developed at Uniformed Services University of the Health Sciences (USUHS). This ecological niche (EM) model was created by experts at USUHS using mosquito location data obtained by GEIS partners in the Republic of Korea (ROK) and Thailand as well as environmental data obtained through GEIS partner researchers at NASA. The original EM model in 2009 was limited to the ROK, but has since been refined and expanded to Southeast Asia and is now available to the general public via VectorMap (www.vectormap.org), another GEIS supported project. This model has been used by the Joint Preventive Medicine Policy Group to help inform JE vaccination policy for US service members, demonstrating the relevance of AFHSC-GEIS products to the US and to global health interests.

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DEMONSTRATION OF A PUSH-PULL STRATEGY INTEGRATING THE AUTO-DISSEMINATION OF A LETHAL AGENT FOR *AEDES AEGYPTI* CONTROL IN IQUITOS, PERU

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Dengue is one of the most important viral diseases in the world. Mosquito control plays an important role in the prevention of infection, due to the lack of an effective vaccine and treatment. A repellent focused push-pull strategy to reduce *Aedes aegypti* inside homes is currently in the proof-of-concept development phase and being evaluated under field conditions. The strategy is based on the use of spatial repellents inside homes (the push component) to discourage adult mosquitoes from entering the treated space and an attractant trap (the pull component) placed outdoors to remove repelled vectors from the peridomestic environment. Combined, the goal is to reduce human-vector contact and the probability of virus transmission. However, it is theorized that the impact of the trap (pull) component could be enhanced by the introduction of a lethal agent (pyriproxyfen) that is auto-disseminated to larval sites by the mosquitoes that have been lured and captured. This push-pull/contaminate-release approach could have a wider effect on vector populations over time. The objective of the current study was to demonstrate under experimental field conditions the feasibility and efficacy of the approach. We report on the use of a mark-release-recapture design to quantify the reduction of *Ae. aegypti* entry into experimental huts using a spatial repellent and the BioGents-Sentinel™ mosquito trap with subsequent counts of mock lethal agent auto-dissemination events in sentinel ovitraps. This information will guide future optimization of the push-pull strategy in preparation for pilot field trials in local homes.

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THE PREFERENCE OF *ANOPHELES FUNESTUS* TO EITHER FEED INDOOR OR OUTDOOR CHANGED WITH TIME AND VECTOR CONTROL INTERVENTIONS IN LUANGWA VALLEY, SOUTHEAST ZAMBIA

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Both personal and community-level impacts of indoor residual spraying (IRS) and long lasting insecticidal nets (LLINs) are mainly dependent upon mosquitoes entering houses, and thus understanding the behaviour of malaria vectors in relation to vector control interventions is fundamental; and is highly variable across the different vector species. The behaviour of *Anopheles funestus*, the primary vector of malaria in Luangwa valley, South east Zambia has been monitored for three consecutive years (2010 - 2012) to evaluate the changes with the ongoing vector control interventions in the area. The main vector control intervention in the first two years was LLINs, but this was complemented with IRS in the third year. Mosquitoes were collected by human landing catches in blocks of houses during the main transmission seasons. At the baseline *An. funestus* equally predisposed to bite indoor or outdoor: the proportions [95% confidence interval] caught indoors were 0.586 [0.303, 0.821]. Increase in endophagy were observed in 2011 and 2012, and the proportions [95% confidence interval] caught indoors were increased to 0.763[0.599, 0.874] and 0.856 [0.437, 0.978], respectively. The mean biting rate was 9.38 bites per person per night at the baseline and this was increased to 17.08 in the second year, and followed by very high reduction to 1.33 bites per

person per night in the third year following the implementation of IRS with pyrimiphos-methyl. The peak biting time was in the late hours of the night, well after people go to sleep and unchanged during the study period. The results are discussed in the context of increased resistance to pyrethroid insecticides and introduction of IRS with pyrimiphos-methyl for malaria vector control in the area.

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ENVIRONMENTAL DETERMINANTS OF RESTING AND FEEDING BEHAVIOR IN MALARIA VECTORS AND ITS IMPLICATIONS FOR MALARIA TRANSMISSION CONTROL

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In recent years the vector species *Anopheles arabiensis* is increasingly responsible for malaria transmission in Africa and reports indicate that in areas of high insecticide treated net coverage, *An. arabiensis* has become the dominant vector species. Unlike its sibling species *An. gambiae* s.s., *An. arabiensis* exhibits much more variable feeding and resting behaviours which will have a strong influence on their transmission capacity and susceptibility to domestic-based vector control measures. This study aims to identify the impact of ecological factors and control measures on the epidemiologically relevant feeding and resting behaviours of *An. arabiensis* within an area of intense malaria transmission, the Kilombero Valley of Tanzania. Here, longitudinal surveillance of vector behaviour has been initiated in four villages where pilot investigations have shown *An. arabiensis* behaviour to vary. Investigation of seasonal variation in the host-seeking (time, and location) and resting behaviour (indoor versus outdoor) of An. arabiensis is being conducted throughout the rainy and the dry season. The relationships between vegetation characteristics, climate parameters as well as the local availability of alternative hosts such as livestock numbers to spatial and temporal variation in mosquito behaviours was tested. Anthropogenic factors including housing type, number of household members and bed nets were also recorded. Ecological niche modelling mixed effects modelling and other multivariate statistical approaches were used to identify the contribution of these factors to the host-seeking time and location of *An. arabiensis*. Results showed *An. arabiensis* behavioural variation over a small geographical range associated with environmental variables. Overall An. arabiensis was found to prefer to bite early and outdoors at high frequency; a strategy which allows them to minimize contact with Insecticide Treated Nets. The vector was also found predominantly resting outdoors. We assessed how these behavioural characteristics impact on current control measures and affect the potential direction for future control measures. Our findings can help to inform decisions about large scale vector control strategies and their expected impact on target vector populations in different ecological settings.

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ALTERNATIVE MOSQUITO VECTOR COLLECTION METHODS IN A SUDAN SAVANNAH AREA OF MALI THAT RECEIVED FIVE MDA ROUNDS FOR LYMPHATIC FILARIASIS ELIMINATION

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To assess the efficacy of two new vector collection methods and their ability to measure vector survival rate, the BG sentinel trap (BGST) and the Ifakara tent trap type C (ITTC) were compared to the more routine Human Landing Catch (HLC) method in 2 villages in Sikasso District in southern Mali that had received 5 annual MDA with albendazole plus ivermectin. Mosquitoes were collected monthly at three sites in each village. The sites in each village were at least 100 meters apart, and the three methods were implemented concomitantly at each site with one BGST trap, one ITTC trap and one HLC unit (one room with two collectors-one inside and the other outside the room). *Culex* spp. were the most common species collected regardless of the method used. Of the 4,500 mosquitoes collected from July to December 2011, 2755 (61.2%) were *Culex spp* and 1,745 (38.8%) were *Anopheles* (*Anopheles gambiae s.l* and *An. funestus*). The yield of anopheles, the vectors of lymphatic filariasis in this region, increased from July to September before decreasing for all collection methods. The total number of anopheles collected by the HLC method was 1,019. This was 34 and 1.5 fold higher than that for the BGST and the ITTC, respectively. Interestingly, there was a significant correlation between the monthly yield of *Anopheles* captured by HLC and the ITTC (r=1, p=0.003) but not by HLC and the BGST (r=0.77, p=0.10) in the village (Bougoula) in which 85.6% of the anopheles were collected. In Boundioba, the correlation between the HLC and the ITTC and the BGST yields were not significant (r=0.61, p=0.19 and r=0.53, p=0.27, respectively). In Bougoula, the *Anopheles* survival rates varied depending on the collection method: 0.92 for HLC, 0.93 for ITTC and 1 for BGST. In contrast, in Boundioba, the survival rates were similar between the methods (0.95, 0.96 and 0.95, respectively). In conclusion, the ITTC appears to be a good alternative collection method to HLC if the vector density is high. This is becoming important as monitoring mosquito vectors and infections rates become increasingly important for the LF elimination process.

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A NEW, EXPOSURE-FREE TRAPPING METHOD TO ASSESS THE BEHAVIOURAL VARIATION IN THE MALARIA VECTOR *ANOPHELES ARABIENSIS*

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The *Anopheles* arabiensis mosquito has become the dominant vector responsible for malaria transmission in the Kilombero Valley, Tanzania and many other regions of Africa. This species exhibits variable host-seeking and resting behaviour. Consequently a more diverse range of trapping tools is needed to permit surveillance of this vector in the varied indoor and outdoor habitats in which it is active. Historically, the most commonly used means to collect anthropophilic malaria vectors was the Human Landing Catch. However, this method is increasingly considered to present ethical issues, as it involves exposing human 'baits' to potentially infectious mosquito bites. Furthermore, this method is labour intensive and hindered by the inherent variability in the attractiveness and skill of collectors. This method is also unsuitable for trapping of zoophilic mosquitoes. Here we evaluated the use of electrocuting grids placed around protected live bait as an exposure-free alternative to the Human Landing Catch. The method offers a more efficient, representative and ethically permissive way for sampling mosquitoes with different behaviours. The novel method makes use of live hosts, animal or human, but ensures they are protected by a mosquito net preventing exposure. The traps can be placed inside houses as well as outdoors. A simple, portable roof protects the trap from heavy rain. This method was evaluated against commercially available insect zappers and Human Landing Catch over 21 nights in Lupiro Village, Tanzania. Results showed a consistent performance of the electric grid traps which compares favourably with insect zappers and Human Landing Catch. The electric grid trap proves to be an excellent alternative sampling tool for malaria vectors which can be applied in many settings. Its flexibility in terms of bait type and trapping location permitted an exposure-free investigation in to the host-seeking behaviour of *Anopheles* arabiensis.

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THE ROLE OF SPATIAL REPELLENTS IN THE CONTROL OF MOSQUITO BORNE DISEASES

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Spatial repellent consumer products have been shown to effectively reduce man-vector contact. They are highly acceptable by most people hence increasingly being used for personal protection. This opens up a niche that can be exploited in order to attain maximum gains in vector borne disease control. Spatial repellents provide a protective bubble within which mosquitoes cannot access humans thus may be effective against mosquito vectors which are now increasingly biting humans outdoors where they remain unprotected. There is need to determine whether repellency as well as modification of mosquito behavior are likely to contribute towards disease reduction. The aim of this study was to quantify the repellency of different doses of transfluthrin (a pyrethroid with a unique chemical formula allowing it to evaporate at ambient temperature and protecting a given space) against mosquitoes outdoors. We used a novel system in which mosquitoes were allowed to respond to different stimuli. This system also enabled the measurement of mosquito responses to stimuli at different distances outdoors. This allowed us to determine the protective effective distance of different doses of transfluthrin. In addition, we conducted experiments to determine whether exposure to different doses of transfluthrin influenced the feeding propensity of mosquitoes as well as the rate of survival of mosquitoes. Results from this study will include dose-response relationships with the main outcome measures being repellency, reduced blood feeding, feeding propensity and survival rate of mosquitoes exposed to different doses. Hence we will provide evidence of the value and role of repellency and mosquito behavioral modification as a highly effective means for controlling disease especially that which is transmitted outdoors.

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SIMULATIONS SUGGEST THAT RELATIONSHIPS BETWEEN MALARIOLOGICAL INDICES ARE STRONGLY MODIFIED BY SEASONALITY

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Evaluating the effectiveness of malaria control interventions on the basis of their impact on transmission instead of their impact on morbidity and mortality is becoming increasingly important as countries move from malaria control to pre-elimination and elimination scenarios. Data on prevalence and transmission are traditionally obtained through resource-intense entomological and epidemiological surveys that become difficult as transmission decreases. This work employs mathematical modeling to examine the relationships between malaria indices to allow more easily measured data, such as serological data and routine health systems data on case incidence, to be translated into measures of transmission. Scenarios with different levels of transmission and patterns of seasonality were created for populations with realistic age-distributions. Simulations of these scenarios were run with an ensemble of models of malaria epidemiology and within-host dynamics. The simulation results show malaria indices are statistically correlated, allowing for estimates of the relationships between these indices for different seasonality profiles. From regression results we take data from one malaria index and calculate the expected range of values for annual average entomological inoculation rate (EIR), prevalence, incidence of uncomplicated and severe episodes, and mortality by age group across seasonal patterns. These results allow for a direct comparison of malaria transmission using data collected with different methods on different indices. For example, when interventions reduce transmission to levels where entomological surveys are no longer feasible, data on incidence can be translated to EIR to measure the effectiveness of the interventions. Although the level of seasonality in transmission is rarely considered in data compilations, modeling results show it can be critically important in determining the relationship between transmission and disease, especially in low transmission areas. Understanding relationships between malaria indices addresses key concerns with the traditional methods of quantifying transmission in areas of differing transmission intensity and sparse data. Although these results still need to be validated, along with seasonal data they can help public health officials detect changes of disease dynamics in a population and plan and assess the impact of malaria control interventions.

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WOLBACHIA INDUCES DENSITY-DEPENDENT INHIBITION TO DENGUE VIRUS IN MOSQUITOES

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Dengue virus is a mosquito-borne pathogen that in recent decades has become a major international public health concern. Increasing problems in pesticide resistance and the lack of drugs and vaccines make it urgent to develop novel strategies for dengue control. *Wolbachia* is a maternally transmitted intracellular symbiontic bacterium that is estimated to infect up to 65% of insect species. The ability of *Wolbachia* to both induce viral interference and spread into mosquito population makes it possible to develop *Wolbachia* as a biological control agent for dengue control. While *Wolbachia* induces resistance to dengue virus in the transinfected *Aedes aegypti* mosquitoes, a similar effect was not observed in Aedes albopictus, which naturally carries *Wolbachia* infection but still services as a dengue vector. In order to investigate the mechanism of this lack of *Wolbachia* -mediated viral interference, we used both Ae. albopictus cell line (Aa23) and mosquitoes to characterize the impact of *Wolbachia* on dengue infection. A serial of sub-lethal doses of antibiotic treatment was used to partially remove *Wolbachia* in Aa23 cells and generate cell cultures with *Wolbachia* at different densities. We show that there is a strong negative linear correlation between the genome copy of *Wolbachia* and dengue virus with a dengue infection completely removed when Wolbacha density reaches a certain level. We then compared *Wolbachia* density between transinfected *Ae. aegypti* and naturally infected Ae. albopictus. The results show that *Wolbachia* density in midgut, fatbody and salivary gland of Ae. albopictus is 80-, 18-, and 23-fold less than that of Ae. aegypti, respectively, while there is no difference in ovary between two mosquitoes. We provide evidence that *Wolbachia* density in somatic tissues of Ae. albopictus is too low to induce resistance to dengue virus. Our results will contribute to our understanding the mechanism of *Wolbachia* -mediated pathogen interference and developing novel methods to block disease transmission by mosquitoes carrying native *Wolbachia* infections.

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SPATIAL DYNAMICS OF ABUNDANCE AND WEST NILE VIRUS INFECTION OF MOSQUITOES IN A SUBURBAN NEIGHBORHOOD

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The spatio-temporal pattern of arboviral tranmission depends on when and where virus amplification takes place, and whether amplification patterns persist. Interpretation of such metrics is hampered by the imprecision of measurement and by use of biologically incompatible spatial and temporal resolutions. In this analysis, we placed 32 light traps in a neighborhood with a strong history of West Nile virus infection in mosquitoes using a paired sample design. This design ensured that

distances between traps represented a range of possible distances. During two summer seasons, we collected host-searching and gravid Culex pipiens mosquitoes, the main vector for West Nile virus in our study area; gravid females collected from 30 gravid traps were tested for virus. Additionally, we have developed a detailed geographic data set for this region, including locations of structures, water bodies and natural areas, and vegetation characteristics. We then used co-kriging and geostatistical modeling, to graph abundance and infection across the study area as a surface taking into account covariates based on the landscape characteristics of the neighborhood. We then tested for spatial autocorrelation at different temporal scales, and grouped the data temporally using periods with similar weather. We compared patterns of infection and abundance at variable temporal scales and in the context of urban structure and investigated the effect of the uncertainty of mosquito infection on the ability for this measure to be used for a robust and reliable vector index. When taking the season as a single unit, we found that mosquito abundance was spatially autocorrelated, and that this autocorrelation persisted over time, both within and between seasons, while mosquito infection exhibited a more spatially independent pattern. A vegetation index improved the model of abundance but not of infection. Overall our approach appears effective in modeling mosquito abundance, but other parameters need to be incorporated for successful modeling of infection.

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CHARACTERIZATION OF THE ENVIRONMENTAL FACTORS AFFECTING SWARMING IN *ANOPHELES GAMBIAE*

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Recent advances in insect's biotechnology has opened several options for malaria control among which the release of genetically modified mosquito rendered refractory to pathogen infection. Another approach that may be effective is based on sterile male release. However availability of these tools does not necessarily guarantee ultimate success. Field and laboratory studies designed to dissect the mating biology of mosquitoes are needed to provide a foundation for predicting the potential utility of genetic control and that this study is addressing.Swarms were surveyed and collected in Vallée du Kou, Burkina Faso in 2011. A complete map of swarm distribution across the study site was constructed and overall 300 swarms were spotted across the village. Swarms strongly responded to specific man-made markers within the village. The number of swarms/compound varied from 3 to 20 and the distribution did not follow normal expectations and exhibited substantial variations suggesting heterogeneity in ecological factors that account for swarm occurrence between compounds. Analysis of the spatial structure of swarms based on Monte Carlo simulations of random distributions indicated that swarms were clustered. Nearest-neighbor distance between swarms was significantly smaller if swarms were randomly distributed over space and the kernel density estimation (KDE) indicated hotspots where most of the swarms aggregate as a response to specific environmental cues. A multivariate analysis allowed identifying a subset of environmental parameters that best correlate to swarm structures and that includes, the number of swarm markers/surface unit, the exposition of the makers to sunlight, the contrast pattern and the openness of the marker to air circulation. These preliminary results have significant meanings towards the achievement of genetically modified mosquito strategies because they suggest that swarms respond to specific environmental cues, hence can be predicted and manipulated.

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EVALUATION OF THE EFFECTIVENESS OF ENTOMOLOGICAL SURVEILLANCE AND VECTOR CONTROL FOR PREVENTION OF DENGUE: SYSTEMATIC REVIEWS OF THE EVIDENCE

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The objectives of the EU FP-7 consortium IDAMS (International Research Consortium on Dengue Risk Assessment, Management and Surveillance) include the development of effective, affordable evidence-based early-warning and outbreak response systems for reducing the impact of dengue. Prior to considering options for new models we reviewed the entomological, epidemiological, meteorological, clinical and economic aspects of dengue surveillance, prevention and control. Vector control remains the only method available to prevent dengue today and entomological indices are still the primary measures of impending risk and intervention success despite doubts about effectiveness in both cases. The findings of reviews of the published evidence on the reliability of entomological surveillance and the effectiveness of dengue vector control tools for dengue prevention are reported here. A defined protocol and standardized approach were followed to review the literature systematically, without restriction on year, location or language. All studies were reviewed by abstract against rigorous inclusion and exclusion criteria. In the case of every included report, two independent reviewers extracted the data. Reports that elicited conflicting interpretations were resolved by discussion. The key findings are summarised and the lessons to be learned and implications for design and implementation of more effective future approaches are discussed.

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AREA-WIDE MANAGEMENT OF THE ASIAN TIGER MOSQUITO, *AEDES ALBOPICTUS*: LESSONS LEARNED

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Aedes albopictus, the Asian tiger mosquito, is the principal vector of chikungunya and a critical vector of dengue viruses. This daytime biting pest is now distributed over much of the eastern quadrant of the continental U.S. all the way north to coastal New York, and often causes the majority of service requests from urban and suburban residents in New Jersey (NJ) and many other states and nations where it has spread. Our objective was to develop an effective strategy for the area-wide control of *Ae. albopictus*, while demonstrating the public health importance and socio-economic benefits of the area-wide control approach. The project was initiated in the spring of 2008 in two counties in NJ and we have tested multiple control and educational interventions, as well as gauged public interest, public participation, and overall costs. This presentation will highlight our major findings regarding the population dynamics and methodology for control of this species in the US.

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HOST SELECTION, DEFENSIVE BEHAVIORS AND FEEDING SUCCESS OF *CULEX QUINQEFASCIATUS* IN EXPERIMENTAL TRIALS

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Studies describing common blood sources of field collected mosquitoes are inconsistent in their description of the host selection behavior of *Culex quinquefasciatus*. Host selection is an important determinant of pathogen transmission, and this knowledge gap in mosquito behavior is limiting our understanding of vector-host contacts and the importance of reservoir hosts in West Nile virus (WNV) transmission. We conducted host-choice experiments under semi-natural conditions to quantify host feeding preference by *Cx. quinquefasciatus* mosquitoes when presented with an array of common passerine hosts: Northern Cardinals, American Robins, Blue Jays, Brown Thrashers, and Gray Catbirds. The experimental design consisted of: 1) a 1.5m x 0.75m x 0.75m enclosure inside of which two bird cages were placed, 2) 30 recently emerged female *Cx. quinquefasciatus* originating from wild eggs, and 3) an infra-red camera recording system. We performed 12 two-bird choice experiments in which we calculated the feeding index for each potential host and tested the null hypothesis of random host selection. We also quantified the number of defensive behaviors exerted by each bird. The blood sources for the 168 mosquitoes that successfully obtained a bloodmeal were assessed by amplifying a fragment of the 16s ribosomal gene using generalist avian primers, sequencing each amplified fragment, and comparing the fragment to reference sequences. Host selection differed significantly from random, exhibiting the following preference structure: American Robins preferred over Blue Jays and over Northern Cardinals, and Northern Cardinals preferred over Brown Thrashers. The most common types of defensive behaviors were those protecting the feet and head, but the number of defensive behaviors did not differ significantly between hosts. Further experiments are needed to determine the role of these defensive behaviors in host selection and feeding success by vectors. Our results indicate a non-random pattern of host selection by vectors that needs to be considered when modeling WNV transmission.

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INTERACTIVE TOOLS FOR IDENTIFICATION OF MOSQUITO AND SAND FLY VECTORS OF INFECTIOUS DISEASES

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Computerized interactive tools to identify mosquito and sand fly vectors of infectious human diseases were developed for various regions of the world (see Walter Reed Biosystematics Unit/WRBU website, www.wrbu.org). Using LUCID programs, WRBU identification keys for mosquito and sand fly vectors and their associated groups included morphological diagnostic characters primarily of the head, thorax, abdomen, legs and wings. Automontage images of diagnostic characters of various insect body parts were attached to each key. Genus and species pages for selected vectors and related groups were developed, including brief basic taxonomy, distribution, bionomics, medical importance, selected references, and detailed photos of habitus and other morphological parts. World catalogs of mosquitoes and sand flies, with updated taxonomy and hierarchic classification were linked to each key. In addition, comprehensive lists of known and potential vectors, and their associated taxonomic information, were included in the WRBU website. New LUCID identification keys were recently developed, namely: African *Anopheles* adult and larval keys (include 140+ species and groups for adult key; 120+ for larval key); South American Culicine mosquitoes (include vector adult and larval keys of *Aedes, Culex, Coquillettidia, Haemagogus, Mansonia, Psorophora, Trichprosophon*); South American Phlebotomine Sand flies (include male and female keys of genera, subgenera, and vector species of *Dampomyia, Evandromyia, Helcocrytomyia, Lutzomyia, Nyssomyia, Pintomyia, Psathyromyia, Psychodopygus, Sciopemyia, Trichophoromyia, Verrucarum* Group). Diagnostic characters, updated taxonomy and related information of new vector identification tools are noted and discussed.

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SRPN2 DEPLETION REDUCES MOSQUITO FITNESS AND BITING FREQUENCY

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The mosquito's immune system is at the vector-pathogen interface and largely determines susceptibility. One consequence of its manipulation can be the reduction in vectorial capacity. Therefore, the mosquito immune system provides potential targets for novel intervention strategies aimed to reduce vector-borne disease burden. Melanization is a powerful immune response in arthropods that leads to encapsulation and killing of invading pathogens. This process renders some mosquito species partially or completely resistant to infection with pathogens of global public health significance. One of its rate-limiting steps of melanization is the activation of prophenoloxidase (PPO), which is controlled by an extracellular proteinase cascade and serpin inhibitors. The molecular composition of this system is largely unknown in mosquitoes with the exception of *Anopheles gambiae* SRPN2 and CLIPB9, which constitute the first known regulatory unit that controls melanization. If uncontrolled, e.g. by the depletion of the inhibitor SRPN2, melanization can kill adult females late in life, and thus potentially reduce the vectorial capacity of *An. gambiae* . This feature makes PPO activation, which is a rate-limiting step in melanin production, a potential target for novel malaria control strategies. Using life table analyses, we determined the consequences of SRPN2 depletion by RNAi on several demographic growth parameters under standard laboratory settings. Net reproductive rate (Ro) was decreased by 29%, while mean generation time was unaffected. As a consequence, doubling time (Td) was moderately increased by 9%. The negative effect on net reproductive rate is largely attributable to a significant decrease in bloodfeeding propensity. Bloodfeeding propensity and survival were disproportionally reduced in older mosquitoes after the first two gonotrophic cycles. As a consequence, the number of potentially infectious bites is at least reduced by 83%. Taken together, these data suggest that SRPN2 constitutes a viable target for novel malaria intervention strategies.

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SPATIAL DISTRIBUTION, SEASONALITY AND BEHAVIOR OF NOVEL MALARIA VECTORS IN THE WESTERN KENYAN HIGHLANDS

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Results from a light trap study carried out in 2010, presented previously, revealed the presence of previously unidentified mosquito species carrying *Plasmodium falciparum* sporozoites in Kisii district in the western Kenyan highlands, an area prone to epidemics of malaria. The majority of these specimens could not be definitively identified to the species level using the commonly used morphological keys, and sequencing revealed that there were no matching published sequences available at ribosomal ITS2 and

mitochondrial CO1 loci. To further morphologically describe these species, study their behavior and investigate their temporal and spatial distribution, mosquitoes collected during both longitudinal and transect studies were identified morphologically and sequenced at rDNA ITS2 and mtCO1 loci. To study spatial and temporal distribution of any novel vectors, monthly pyrethrum spray catches were made to sample indoor resting mosquitoes in 10 villages across Kisii Central, Kisii South and Rachuonyo South districts over an 18-month period between 2009 and 2011. To determine host-seeking activity and peak biting times, mosquitoes were collected from light traps set up indoors and outdoors over a 12-month period from June 2011. To fully describe larval and adult stages, a wide range of larval breeding sites were sampled across Rachuonyo South in November 2011 and 400 *Anopheles* larvae were reared to obtain adults with associated larval and pupal exuviae. For specimens found to have novel unpublished sequences, taxonomic studies were conducted to establish the identity of the species. Results on the presence of unusual species, their associated behaviors, spatial distributions and seasonality are presented.

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INVESTIGATING THE ROLES OF *ANOPHELES GAMBIAE* G PROTEIN-COUPLED RECEPTORS IN GUSTATION

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Vector-targeted control strategies remain our most effective tools for reduction of malaria transmission and incidence. However, the threat and continuing increase in insecticide-resistance motivate discovery of novel insecticides. G protein-coupled receptors (GPCRs) are well known as one of the most "druggable" targets in many organisms. Numerous GPCRs mediate developmental, sensory or other physiological pathways that can greatly impact vectorial capacities of *Anopheles gambiae* and other malaria vectors. Gustatory GPCRs are central to the ability of insects to identify foods, including sugars, and detect noxious compounds in the environment. We are investigating the abilities of *An. gambiae* to detect various sugars and noxious compounds when given a choice between sugar meals, or between a sugar meal and a sugar/compound meal. Dye-labeling of meals enables colorimetric detection of intake. By using RNA interference to independently knockdown individual gustatory receptors, we will identify specific GPCRs required for sugar and noxious compound gustation. We have begun by analyzing sugar preferences among glucose, sucrose, fructose, galactose and mannose. Among these sugars, preliminary results imply mosquitos prefer glucose and sucrose. Initial data suggests that mosquitos exhibit strong aversion to berberine, a canonical noxious compound used in insect gustatory preference assays. We will report further progress in the analysis of sugar preference and noxious compound sensing in *An. gambiae*, and initial RNA interference results characterizing requirements for different GPCRs in sugar and noxious compound gustation. The overall goal of this project is to understand the functional roles of the mosquito gustatory GPCRs and exploit this system to enhance development of sugar-meal based, vector-targeted interventions that will decrease vectorial capacity of *An. gambiae* and other malaria vectors.

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OUTBREAK CLUSTERS OF *FASCIOLA HEPATICA* IN ARGENTINA

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Fasciola hepatica is a trematode responsible for the human disease fascioliasis. It differs from other human liver flukes in that it is present worldwide. Fascioliasis is transmitted through the ingestion of the metacercaria stage parasite found in infected vegetables such as watercress. Although it generally presents as chronic indolent individual infections, outbreaks of acute fasciolasis can occur in endemic areas related to ingestion of a contaminated food item. Despite the high prevalence of *F. hepatica* in many areas, its importance has been largely neglected. We investigated clusters of *F. hepatica* which occurred in four family groups in rural Argentina. A total of 34 confirmed cases of acute fascioliasis from 4 different families (15, 6, 8, and 5 affected members) were investigated. Three of the four families were residents of endemic areas, while a fourth family vacationed in an endemic area. All members from the 4 families reported ingestion of watercress. The most common clinical symptoms were right upper quadrant pain and fever, present in all subjects. All cases had elevated liver transaminase tests as well as absolute eosinophilia. *Fasciola* ova were detected in stool samples in all 34 subjects. Serology was performed in 16 subjects and was positive in 13 (81%). Abdominal ultrasound (performed in 22 subjects) showed diffuse hepatomegaly in 19 cases (86%). Twenty patients were treated with intravenous emetin and 14 patients with triclabendazole. Of 29 subjects who presented for post-treatment stool ova and parasite examination at 60-days, 26 (90%) became negative, with the remaining 3 patients becoming negative by 90-days post-treatment. In the triclabendazole group, 2 subjects had relapse with one subject needing repeat treatment and another subject needing two additional treatments. In the emetin group one patient needed repeat treatment, however three individuals developed hypotension as a treatment complication requiring hospitalization. *F. hepatica* remains an under-recognized infection. In fact, most subjects reported here did not seek medical care since they had only mild clinical symptoms and were only detected through the outbreak investigation. There is a need for increased education and awareness of fasciolasis in patients living and traveling to endemic areas.

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SCHISTOSOMIASIS AND SOIL TRANSMITTED HELMINTHIASIS IN KAÉDI, SOUTHERN MAURITANIA

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We report findings of a cross-sectional parasitological study on schistosomiasis and soil transmitted helminthiasis among school aged children since the establishment of irrigation schemes in Kaédi, located at the confluence of the Gorgol and Senegal Rivers in Southern Mauritania. Stool and urine samples were obtained from 246 children between the ages of 5 to 15 years recruited from randomly selected households followed by administration of a parental questionnaire. Urine samples were analysed the same day using the centrifugation method, while stool samples were collected early the next morning and analyzed by the Kato-Katz technique. We found a low prevalence of *Schistosoma haematobium* (3.7 %) with a mean infection intensity of 38 eggs per 10 mls of urine, while no cases of S. mansoni were detected in all participants. Only one participant was infected with a soil transmitted helminth (*A. lumbricoides*). Working in the rice paddies during the annual flood recession (Oualo farming) was the strongest predictor of infection intensity among participants when we fitted a zero-inflated negative binomial (ZINB) model for egg counts with potential confounding factors controlled for in multivariate analyses.Prevalence of *S. haematobium* seems to have decreased compared to last estimates before the establishment of the irrigation schemes (14 %). These findings highlight a need for an integrated surveillance system aimed at transmission control targeting various aspects of the transmission cycle *S. haematobium*.

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DEVELOPMENT OF A RECOMBINANT PROTEIN VACCINE AGAINST *SCHISTOSOMA MANSONI* INFECTION USING CATHEPSIN B AND PEROXIREDOXIN 1 ANTIGEN

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Schistosomiasis is a fresh-water-borne parasitic disease caused by trematode worms of the genus *Schistosoma*. Due to its morbidity and mortality, Schistosomiasis is the most important helminth infection. The pathology of the disease is due to egg deposition, by the female worm, which will trigger an immune reaction and consequently cause progressive damage to the organs. The lack of therapeutic drugs and preventative measures, as well as the high disease burden caused by the infection are justifications for developing a vaccine against schistosomiasis. The development of a recombinant protein vaccine against this parasitic disease has the potential to contribute a long-lasting decrease in disease spectrum and transmission. Furthermore, it would relieve some of the concern surrounding the potential emerging resistance to praziquantel; the drug which is solely being used to treat the infection. Our group has chosen to focus on the *S. mansoni* antigens Cathepsin B and Peroxiredoxin 1 (Prx1) as vaccine candidates. It is hypothesized that immunization with either recombinant Cathepsin B or recombinant Prx1 in the presence of an adjuvant can elicit protective immunity against *Schistosoma* infection. The objective of this research project is to develop a safe recombinant protein vaccine against schistosomiasis that will stimulate an optimal immune response which will prevent pathology. Upon cloning, expressing, and purifying the proteins of interest, mice were firstly immunized with recombinant Cathepsin B in the presence of either synthetic oligodeoxynucleotides (ODN) containing unmethylated CpG dinucleotides or Montanide ISA 720 VG. The mice received two booster injections following the first immunization. The vaccine formulations were not toxic, and all of the mice survived until the end of the study. The vaccine elicited a pronounced production of *S. mansoni* Cathepsin B specific antibodies whereas no antigen-specific antibodies were found in the control animals. Splenocytes proliferated in response to Cathepsin B and produced elevated levels of Th1, Th17, and inflammatory cytokines. These results highlight the potential of *S. mansoni* Cathepsin B as a promising vaccine candidate for schistosomiasis. The investigation concerning Prx1 is ongoing.

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PROJET-CREVETTE: AN INTERNATIONAL COLLABORATION TO REDUCE PARASITIC DISEASE, RESTORE THE ENVIRONMENT AND IMPROVE LIVELIHOODS IN WEST AFRICA THROUGH AQUACULTURE

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"Projet-Crevette" is a collaborative research project to investigate novel ways to combat the spread of schistosomiasis in rural Africa. In 1986, the Diama Dam was built on the Senegal River in West Africa in order to stabilize river flow, reduce drought conditions, and support a growing agriculture industry. Within 5 years after dam construction, schistosomiasis spread rapidly, leading to an epidemic that has persisted until today, with > 90% prevalence among some rural villages along the river. River prawns, which at one time were voracious predators of snails, have recently been decimated in the Senegal River due to habitat loss above the Diama Dam. Thus, Projet-Crevette aims to develop an aquaculture program to supplement prawn reproduction and re-introduce native river prawns to the Senegal River basin as predators of snails that carry schistosome parasites. Projet-Crevette has monitored the distribution and abundance of prawns, snails, and schistosome parasites at 11 sites throughout the Senegal River Basin over the course of one full year during 2011. This baseline data will pave the way for development of an innovative parasite-control strategy that promises to simultaneously combat disease transmission, restore the environment, and improve livelihoods by restoration of an artisanal prawn fishery.

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COMPARISON OF USEFULNESS OF *SCHISTOSOMA MANSONI* SOLUBLE CERCARIAL ANTIGENS AND SOLUBLE EGG ANTIGENS IN ELISA FOR SERODIAGNOSING SCHISTOSOME INFECTIONS

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Diagnosis of schistosomiasis is problematic since no method is yet available that gives both 100% sensitivity and 100% specificity. The traditional, most widely used method is microscopy, but because of inherent insensitivity this technique often wrongly diagnoses patients as uninfected. Use of serological assays involving detection of specific antibodies is now increasing since the putative sensitivity of these tests is much higher than that of other alternative methods of diagnosis. They are routinely used in travellers' medicine clinics where often only light infections are encountered and which microscopy is not sensitive enough to detect. ELISA incorporating schistosome soluble egg antigens (SEA) is often the antibody-detection test of choice. The use of SEA-ELISA for diagnosis of schistosomiasis in developing countries is however restricted since SEA is relatively expensive to produce. We have investigated whether a cheaper alternative prepared from *S. mansoni* cercariae, namely cercarial transformation fluid (SmCTF), could potentially replace SEA in ELISA. Our results demonstrate that SmCTF performs equivalently to *S. mansoni* SEA for the detection of both anti-*S. mansoni* and anti-S. haematobium antibodies, and that SmCTF is even comparable to S. japonicum SEA for schistosomiasis japonica. These results have laid the foundations for the development of a rapid diagnostic test (RDT) incorporating SmCTF for detection of anti-schistosome antibodies. Such a RDT would meet all the ASSURED criteria for diagnostic tests, particularly with regard to being Affordable and User-friendly, and could thus be useful in the developing world where the majority of the disease burden lies.

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IN VITRO, HUMAN EOSINOPHILS DOWN MODULATE PERIPHERAL BLOOD MONONUCLEAR CELLS RESPONSES TO *SCHISTOSOMA MANSONI* ADULT WORM ANTIGENS

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Eosinophils have been regarded as terminally differentiated non-replicating effector cells observed in a number of health disorders including parasitic infections and allergic diseases where they play a beneficial role in the host defence against helminth infections or cause a harmful inflammatory response respectively. In schistosomiasis eosinophils have been associated with direct or indirect killing of schistosomula. However, there is growing evidence that eosinophils can play an additional immunoregulatory role in both adaptive and innate immunity to parasitic infections. Here we report results of a study, using samples from *Schistoma mansoni* infected individuals, in which we investigated the effects of co-culturing human eosinophils with peripheral blood mononuclear cells (PBMC)

on *in vitro* cytokine production in response to *S. mansoni* adult worm antigen. PBMCs obtained from 26 *S. mansoni* infected adults were examined for cytokine responses to *S. mansoni* adult worm antigen (SWA) when stimulated alone or when enriched with autologous eosinophils. Production of IL-4, IL-5 and IL-13 was lower (p=0.017, 0.018 and <0.001 respectively) in PBMC+eosinophil cultures than in PBMC-only cultures stimulated with SWA. IL-13, IL-10, IFN and TNF were released in eosinophil-only cultures but none of these cytokines produced by the eosinophils showed a significant association with the observed eosinophil-induced drop in cytokine responses of PBMCs. This preliminary study shows that eosinophils can exert a down-modulatory effect on schistosome specific responses. The mechanism of this immune-modulation remains to be elucidated.

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SCHISTOSOMA HAEMATOBIUM RECOMBINANT PROTEINS AS A VACCINE CANDIDATE FOR HUMAN SCHISTOSOMIASIS

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In Sub Saharan Africa human schistosomiasis is largely caused by *Schistosoma mansoni* and *S. haematobium*. The current strategy for controlling morbidity of the disease is through mass drug administration using praziquantel, the drug of choice. This dependency of using only one drug can possibly induce praziquantel resistance of the parasite and could render this method of intervention ineffective. This has therefore necessitated the urgency for the development of a vaccine to combat the disease. With the focus of most schistosomiasis vaccine developments on *S. mansoni* parasite, it could be challenging for identified putative vaccine candidates to elicit the required immunological responses in S. haematobium-endemic communities in African populations, where the disease is caused by either S. haematobium or in regions of co endemicity. If this condition arises, it could lead to the detriment of full potential of *S. mansoni* vaccine candidates. It will therefore be complementary for vaccine design efforts to strive into proteomic and immunology of S. haematobium counterpart as well. Here we discovered 17 orthologs hits (5 Tetraspanin proteins, 5 CD59-like proteins, 2 MEG-8 proteins, 2 Saponin proteins, 1 FOG precursor, 1 Stomatin-related protein) from *S. mansoni* sequences. This bio-informatic lead analysis was performed using tBLASTn search of Wellcome Sanger S. haematobium ESTs libraries against 52 *S. mansoni* vaccine candidates (with e-value 3.90e-30-3.00e-110 and identity value 56%-93%). These 17 orthologs were characterised by non-allergen, had no human orthologs and was expressed during vertebrate infections (definitive host). 3 of such orthologs (1-Tetraspanin, 1-MEG and 1-CD59 like proteins) were produced as recombinant proteins and being used as lead proteins towards the discovery of S. haematobium vaccine candidates. In conclusion, if vaccine candidates are identified from S. haematobium, it will not only mark a crucial milestone in terms of vaccine development for disease but will drastically facilitate the reduction of schistosomiasis in Africa.

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VACCINATION WITH RECOMBINANTLY EXPRESSED GLYCAN ANTIGENS FROM *SCHISTOSOMA MANSONI* INDUCES GLYCAN-SPECIFIC ANTIBODIES AGAINST THE PARASITE

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Schistosomiasis caused by infection with the parasitic helminth *Schistosoma mansoni* is a major global health problem due to inadequate diagnosis and treatment, and lack of a vaccine. Vaccine candidates have failed due to the worm's complex architecture and life cycle, exquisite modulation of host immunity, and our incomplete understanding of antigens targeted during infection. The immune response to schistosomes is primarily directed against glycans, rather than protein antigens, and evidence suggests that glycans could be valuable diagnostic markers and protective vaccine targets. The di- and tri-saccharide motifs LacdiNAc (GalNAcβ1,4-GlcNAc; LDN) and fucosylated LacdiNAc (GalNAcβ1,4-(Fucα1-3)GlcNAc; LDNF), are expressed throughout the *S. mansoni* life stages and are densely distributed among many glycoconjugates in monomeric form or as repeating units (poly-LDNF). Such determinants are lacking in mammals. LDN and LDNF are antigenic in several *S. mansoni*-infected mammals, yet, how to make such glycans antigenic in the context of a defined vaccine has remained elusive. We have developed a recombinant expression system in which a Chinese Hamster Ovary (CHO) cell mutant termed Lec8 expresses repeating forms of LDN (Lec8GT) and LDNF (Lec8GTFT) abundantly on its glycoproteins. Immunizing mice with these cells induced glycan-specific antibodies and a sustained booster response. The Lec8GTFT anti-sera were cross-reactive with *S. mansoni* and displayed exquisite specificity for particular presentations of LDNF antigen on glycan microarrays. We are currently investigating the cellular mechanisms supporting this anti-glycan antibody production, including T-cell dependence and memory B cell compartments, and we are using glycan microarrays to more specifically define the structures that comprise antigenic LDNF in *S. mansoni* infection. Our recombinant expression system has proven to be successful at invoking antibodies to the antigenic glycans of *S. mansoni*, and can be adapted to study many other pathogens and novel glycan antigens for use in vaccines and diagnostics.

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HISTONE MODIFYING ENZYMES AS PUTATIVE DRUG TARGETS FOR SCHISTOSOMIASIS

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Histone modifying enzymes (HMEs) play key roles in the regulation of chromatin modifications. Furthermore, aberrant epigenetic states are often associated with human diseases, leading to great interest in HMEs as therapeutic targets. The availability of the genomic data of three *Schistosoma* species provides an opportunity to identify new drug candidates against schistosomiasis. In this work, we have identified and characterized all enzymes involved in histone acetylation and methylation that include: histone acetyltransferases (HATs), deacetylases (HDACs), methyltranferases (HMTs), and demethylases (HDM). We analyzed the predicted proteomes of the parasites in order to identify and classify the HMEs through computational approaches, mainly by using Hidden

Markov Model profiles. We were able to identify around 60 HMEs with some variation within the three *Schistosoma* species. From the identified enzymes, 24 were tested individually as therapeutic targets using RNA interference in cultured larval stages (schistosomula) to invalidate each corresponding gene. Although, gene knockdown of up to 90% could be achieved, no phenotype could be observed after 7 days of dsRNA exposure. Loss of motility could be observed as a phenotype for two HDMs after 30 days of dsRNA exposure. In addition, in order to assess the role of genes in the presence of the host environment under immunological pressure, knockdown parasites for four HMEs (HDAC8, KDM1/ KDM2 and PRMT3) were tested *in vivo*. A significant reduction of worm burden (50%) could be observed in mice infected with knockdown parasites for HDAC8 when compared to unspecific control. Finally, egg count was significantly reduced in mice livers for all tested HMEs. In conclusion, our work improved the functional annotation of over 20% of *S. mansoni* HAT and HDAC proteins. Parasites with reduced levels of HDAC8, KDM1/KDM2 and PRMT3, seem to diminish the oviposition and ability to survive (for HDAC8) in the host milieu, indicating that these enzymes could be good target candidates for drug development.

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SCHISTOSOMIASIS COLLECTION AT THE NATURAL HISTORY MUSEUM (SCAN)

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The Natural History Museum, London, maintains one of the largest biodiversity collections in the world and is a WHO Collaborating Centre for the identification and characterisation of schistosomes and their intermediate snail hosts. *SCAN*, the Schistosomiasis Collection at the Natural History Museum is a new initiative to make existing schistosome and snail host specimens available to the research community, facilitate new monitoring and research projects by providing a sample repository, and make samples available to the research community. Many of our archived schistosome specimens, representing a legacy of decades of field sampling, are suitable for molecular genetic applications, and new schistosome collections, concentrating on the accessible larval stages, are being archived using ambient DNA storage methods. Monitoring and research projects that accompany schistosomiasis control programmes generate specimens and data used to fulfil the objectives of the project. These specimens can also have a value beyond these immediate requirements as new questions emerge, tools improve, or wider comparisons become possible. To facilitate future use, an infrastructure to consolidate, maintain and distribute them is needed. SCAN aims to provide this infrastructure. At present, working primarily with SCORE, the Schistosomiasis Consortium for Operational Research and Evaluation, SCAN is providing support as follows: provision of a central specimen repository for several SCORE sub-projects; assistance with collection and transportation; data entry and consolidation; methods development. Additional to the benefits of an archive, SCAN's collection management priority has immediate advantages for collection, training and data curation activities within SCORE sub-projects. The success of SCAN and depends on the support and trust of control teams, researchers and funding agencies.

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REDOX BIOLOGY AND DRUG DEVELOPMENT FOR SCHISTOSOMIASIS

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Schistosomiasis remains an important neglected disease with 200 million infected individuals. Individual treatment and large-scale control campaigns rely primarily on the use of praziquantel, the only available drug for schistosomiasis treatment. There is concern that praziquantel resistance will evolve and, in the absence of alternative therapies, control measures will be imperiled. Enzymes in the redox pathways of schistosomes have been found to be suitable targets for schistosomiasis drug development and schistosome antioxidant enzymes have been shown to be essential and druggable proteins. Of particular interest is thioredoxin glutathione reductase (TGR), which plays a central role as a multifunctional protein entirely providing the activity of several distinct enzymes present in the human redox network. Therefore, TGR is a redox bottleneck in schistosomes. Oxadiazole 2-oxides have been identified as TGR inhibitors, acting through both nitric oxide production resulting from TGR activity and TGR inhibition. We will present results defining the role of nitric oxide in the action of oxadiazole 2-oxides and other nitrosating agents both in the local context of TGR S-nitrosylation and global context of other schistosome proteins susceptible to modification by nitric oxide. In addition, a rescreen of the NIH Chemical Genomics Center compound library has identified many new classes of small-molecule TGR inhibitors. Mechanisms of action, activities against *ex vivo* parasites, and structure-activity relationships of these compounds will be discussed.

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SIGNIFICANCE OF THE SO-CALLED "APO-AMEBOCYTE PRODUCING ORGAN" IN *BIOMPHALARIA GLABRATA*

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Internal defense against microorganisms are performed in mollusks by a single cell type: the hemocyte or amebocyte. Their place of origin in *Biomphalaria glabrata* has nowadays become a matter of controversy. Initially, the hypothesis maintained by several authors was that the amebocytes had a multicentric origin. However, more recently it has been postulated that *B. glabrata* amebocytes are instead formed within a central special organ. The initial argument for the Amebocyte Producing Organ (APO) being considered as the locale of origin for hemocyte production in *B. glabrata* was the finding of hyperplasia and mitoses in its cells during the course of *Schistosoma mansoni* infection. The present investigation was concerned with a morphological analysis, with histological, immuno-histochemical, morphometrical, and ultra-structural findings, from the so-called *B. glabrata* APO. Its structure was identified as a collection of epithelial basophilic cells, disposed on one-cell-thick layer or in small round collections, covering a small area of the pericardial surface in the reno-pericardial region. Sometimes it vaguely resembled the epithelial component of the vertebrate juxta-glomerular apparatus of the kidney. During our studies, mitoses were only occasionally found, either in normal or infected mollusks. Also our quantitative studies failed to demonstrate the presence of APO cellular hyperplasia, either in normal or schistosome-infected *B. glabrata*. Therefore, our findings did not provide evidence in support of the so-called APO being considered the central organ for hemocyte production in *B. glabrata*. Multi-focal proliferation of hemocytes was found in many other areas of the mollusk during *S. mansoni*-infection. By contrast, several structural details from the "APO" region in *B. glabrata* were found to be consistent with the suggestion that it is indeed a filtration organ, more related to the kidney, as evidenced in other species of mollusk, such as *Lymnaea truncatula,* rather than bone marrow.

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ASSESSING THE HEALTH IMPACT OF IMPROVED RURAL SANITATION: DESIGNING AND CARRYING OUT A CLUSTER-RANDOMIZED, CONTROLLED TRIAL IN ORISSA, INDIA

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Despite progress on other MDG targets, sanitation coverage continues to fall behind with 2.6 billion people still lacking access to even basic sanitation. More than one billion people still practice open defecation, including an estimated 636 million in India alone. One possible reason for the slow progress in sanitation is the lack of clear, compelling evidence about the effectiveness and cost-effectiveness of sanitation. To date, there is no randomized controlled trial of sanitation interventions to prevent diarrhoea diseases. We describe the design and execution of a large-scale study that seeks to help close the evidence gap on rural sanitation in low-income settings. Using a cluster-randomized trial design, the study aims to assess the effectiveness of a project by Water Aid India to promote the construction and use of individual household latrines in accordance with the Indian Total Sanitation Campaign. The study population consists of 100 villages (about 12,000 people) in a costal district of Orissa, India. The main objective of the trial is to assess whether improved sanitation reduces diarrhoeal and helminth infection among young children. The presentation will emphasis five additional aspects that we believe necessary in designing evaluations of sanitation interventions: (a) comprehensive process evaluation_carefully documenting the manner in which the intervention is actually implemented rather than intended or reported by the program implementers; (b) documenting uptake_the actual use of the intervention by the target population_since there is widespread evidence that latrine use is sub-optimal in India; (c) assessing whether the intervention has actually reduced exposure_a condition to securing health outcomes; (d) spatial analysis and spill-over effects from sanitation interventions, and (d) longer-term assessments due to (i) the longer time required to implement the intervention, (ii) the potential persistence of excreta-related pathogens in the environment even after the widespread uptake of an effective sanitation intervention, (iii) the need to investigate longer-term changes in uptake, and (iv) the need to follow whether safe and effective pit-emptying is underway.

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STUDIES ON THE PRESENCE OF *CRYPTOSPORIDIUM SP* AROUND WATER TREATMENT PLANTS THAT SUPPLY WATER TO GREATER ACCRA REGION OF GHANA

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Cryptosporidium sp a Protozoan has emerged in recent times as zoonotic parasite. These parasites of the Apicomplexan family were found in association with diarrhea in calves and are water-borne. The organism is second only to rotavirus as a causative agent of diarrhea in newborn calves and infants. As such, it is a potentially serious contaminant in water where cattle graze. In order to estimate the human health risk in cattle rearing areas around water treatment plants, we measured the prevalence of Cryptosporidium oocysts in the fecal matter from four cattle ranges upstream in Joma near the Densu Dam at Weija in the Ga South Municipality and Kpong in the Lower Manya District all of Southern Ghana. The Modified Ziehl-Neelsen staining technique (MZN) for *Cryptosporidium* oocysts was used. Of the 320 fecal samples for each species screened, 63 (19.7%) were positive for *Cryptosporidium*. Prevalence was higher in calves younger than three months of age, as compared to weaned calves and adults. Oocysts were detected in both diarrheic and non-diarrheic samples, with a significantly higher prevalence ($p < 0.05$) of oocysts shedding in diarrheic samples.

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LACK OF EDUCATION OF HOUSEWIVES CONCERNING THE TRANSMISSION RISKS FACTORS OF TYPHOID FEVER IN THE DR CONGO

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DRC is still characterized by a critical socioeconomic situation impacting negatively the quality of health services offered to the population, createing a permanent problem of hygiene and prevention of diseases. Every year the Country faces various epidemic outbursts of avoidable diseases such as typhoid fever (TF).In addition, more than 50 % of Congolese women are illiterate, whilst it is established that the mother's education level is the most determining factor for the family's health and nutritional status. A proper washing of the hands especially before preparation of food, before the meal, after the toilet; access to healthy source of water; safe elimination of human excreta, are the key factors to help reduce the frequency of diseases with oro-faecal transmission. To assess this assertion, we conducted a Community based survey from June 1st, to August 31st, 2008 to assess the knowledge and practices relative to the prevention of TF transmission by questioning 500 domestic women of the City of Bunia in the northeast of the RDC.The Study used a randomization method for the selection of the Housewives by Quarter of the City of Bunia (40% subset of the Quarter population) Of the 500 women interviewed: 288(56.7%) were 20-24 years old; 325(65%) unemployed; 261(52.2%) have not attended school or only primary school; only 198(39%) have some sufficient knowledge of the transmission of TF; 267(53.40%) have little or no knowledge of the good qualities of drinking water ; 265(53.00%) are unaware of the rules of food hygiene; only 137(27.00%) practice correctly the washing of the hands; all households use unsafe sources of drinking water and 380(78.8%) among them do not treat the drinking water (boil or treat with Chloramines); 202(42.60%) of women use non hygienic latrines and 85(17%) of households do not have latrines at all. The promotion of hygiene and specifically education of housewives remain fundamental in the improvement of national educational strategies in the DRC.This finding might be relevant to all Stakeholders involved in the fight against TF.

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AN UNUSUAL PARTNERSHIP TO ENSURE SAFE DRINKING WATER TO THE RURAL POPULATION IN INDIA

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Water treatment at the point-of-use (POU) can reduce diarrhea caused by waterborne pathogens by 30 to 50 % (WHO 2007). The goal of the project was to demonstrate a comprehensive strategy aiming at increasing use of POU water treatment methods among poor urban and rural populations and thereby reduce childhood diarrhea in the state of UP in India. The at-scale goal was to achieve 30 % rural and 40 % urban use of an effective POU method. By January of 2009, the partnership between POU manufacturers and NGO partners was formalized through MOUs. The project reached 674,064 households residing in 1120 urban slum areas and 1350 rural villages in UP. A quantitative study of 1400 households at baseline, showed only 2.5 % of households (4.1 % urban and 1.1 % rural) reported ever using a POU method promoted by the project (boiling, disinfection products, or filtration). In contrast, the outcome evaluation found very high rates of POU use in both the intervention and comparison areas, with 96.8 % of intervention households reporting they had ever used a recommended POU method, along with 71.0 % of households in the comparison areas. The biggest difference between intervention and comparison districts was in the use of chlorine liquid for

disinfection (56.9 % versus 0.3 %). No difference was found in the use of water filters (about 7 % in both areas). Among urban households, 50 % reported current use of chlorine tablets, vs. 3 % of rural households. Conversely, 60 % of rural households reported current use of liquid chlorine, vs. 11 % of urban households. This clear preference for different products cannot be explained by any difference in intervention approach, and bears further investigation. An engaged commercial sector was able to reach a substantial new market by partnering with NGOs and micro-finance institutions. NGOs can be trained to become effective product demonstrators and micro-distributors. The long-term viability of NGO POU product distribution should be monitored. Commercial partners are now expanding the model in other states in India

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DO THE CHILDREN GETTING WHAT DO THEY NEED TO WASH HANDS IN SCHOOL? EXPERIENCE FROM BANGLADESH

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Schools are common sites for the spread of gastro-intestinal and respiratory diseases. There are a variety of hygiene interventions linked to hand washing, respiratory hygiene, sanitation and water quality, which have shown some success in preventing and /or reducing these diseases. However, little research has been done on the feasibility and effectiveness of these water, sanitation and hygiene interventions in school settings in Bangladesh. The objective of this study was to understand the current practice regarding hand washing and facilities needed to wash hands from primary school children in a low income country like Bangladesh. The study used data from in-depth interviews and observations conducted with purposively selected school children in Bangladesh. The interviews were conducted with a topic guide line developed based on existing literature and in consultation with study investigators. Transcripts were processed using a thematic-analysis approach. Major findings indicated that increasing hand washing in low resource setting is a complex process, it included that after giving knowledge, knowledge increased but lack of hand washing facilities in school premises influence the practice of hand washing in school. A greater number of informants stated that availability of resources like soap and water supply is important to keep the practice of hand washing in school. Most of the schools do not have fund and capacity to supply soap and water. Children are motivated to wash hands due to school hygiene program but cannot practice their knowledge as schools are not able to supply those facilities. School Authority suggested for better communication with government before implementing intervention so that government can help to generate fund to continue the program.

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PRELIMINARY ASSESSMENT OF THE POTENTIAL EFFECTIVENESS OF WATER FILTERS TO REDUCE DIARRHEAL DISEASE BURDEN IN CHILDREN YOUNGER THAN FIVE YEARS OLD IN A PACIFIC ISLAND NATION

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A novel household water treatment device was proposed for use in the Kiribati, a small, remote pacific island nation. In collaboration with the Kiribati Ministry of Health and the WHO (South Pacific) we conducted a field study to understand the extent to which water-borne diarrheal disease is an important health issue, and whether a domestic water filter used in this setting is likely to reduce the incidence of diarrhea. As far as we are aware, this is the first investigation of its kind in any pacific island nation.In this field study of 97 randomly selected households of 802 individuals we found that 7% of participants, and 25% of children under 5 years old had experienced diarrhea in the past month and 7% of children under 5 had experienced diarrhea in the past week. Participants reported high levels of open defecation (59% children) combined with low knowledge of the danger of childrens' feces and low levels of handwashing, especially after defecation and the handling of childrens' feces. It is highly likely that contamination from hands and flies goes on to contaminate food and individuals directly leading to high levels of 'water-washed' (rather than water-borne) endemic diarrhea. Most individuals we interviewed (86%) reported that their household normally boils their water for drinking. Water samples were highly contaminated, and there was not a statistically significant difference in fecal coliforms between source water and drinking water. In households that boiled their drinking water, it was less contaminated than the source water in only half the samples, suggesting that significant recontamination occurs following boiling, this would likely happen following filtration. Almost all (91% of households) store drinking water and only 24% use safe storage containers, while the remainder access drinking water by dipping dirty cups, vessels, and hands into the water container. Information on behaviour and water quality indicates that the transmission of endemic diarrhoea is likely to be through many pathways other than drinking water, and even treated water is highly susceptible to recontamination. Filtration as a form of household water treatment is likely to have limited effect in this setting.

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DEVELOPING A SOCIAL ECOLOGICAL MODEL FOR *VIBRIO CHOLERAE* TRANSMISSION DYNAMICS IN HAITI: IMPLICATIONS FOR CONTROL STRATEGIES AND PUBLIC POLICY INTERVENTIONS

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An evaluation of Catholic Relief Service's (CRS) post-earthquake cholera education programming in Haiti was conducted in June 2011 to evaluate the efficacy of their social marketing efforts for cholera prevention. A Knowledge, Attitude, and Practice (KAP) survey implemented throughout Haiti provided cholera incidence data as well as social and behavioral data that indicate sources of disease transmission and water contamination. Evaluation results indicate that there remain gaps in practices such as hand washing and open defecation. These results highlight the importance of monitoring of incidence data and surveillance in countries where poor infrastructure and a lack of proper sanitation facilities necessitate changes in routine behavior to prevent outbreak. Monitoring and prevention, coupled with mathematical compartmental models of transmission dynamics, would enable prediction of future cholera outbreak risk per commune and also would enable the Haitian Ministry of Health, HSPP, and the population to take preventative measures well in advance. Thus, a novel SIR-type social ecological model for *Vibrio cholerae* transmission dynamics in resource-poor settings has developed, incorporating behavioral data from the KAP evaluation. Furthermore, the behavioral and ecological factors that have been integrated into the base model ensure greater predictive ability at the commune level when the model is back-fitted to prior incidence data. Such models hold the key to affecting control strategies and public policy interventions in ways that ensure a given population is prepared for a potential outbreak when conditions are ideal.

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CHOLERA OUTBREAKS IN URBAN BANGLADESH IN 2011

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In February 2011, an outbreak of severe diarrhea was reported at a tertiary medical college hospital campus in Bogra District in northwest Bangladesh. In April 2011, a similar outbreak was reported at 3 urban communities in the northeastern district of Kishorganj. We investigated these outbreaks to determine the etiology and pathways of transmission. We visited the tertiary hospital in Bogra and the secondary hospital serving the affected communities in Kishorganj. We listed the admitted cases of severe diarrhea (passage of ≥ 3 loose stools per day) from the affected areas. We interviewed the admitted cases, physically examined them and collected rectal swabs in bacterial transport media to test for enteric pathogens including *Vibrio cholerae*. We visited the affected communities to explore the water supply and sanitation. We collected water samples from selected cases' household taps, tube wells and central pumping stations to test for microbes including *Vibrio*. We identified 21 cases from Bogra and 84 cases from Kishorganj. The median age was 23 years in Bogra and 21 years in Kishorganj. There were no reported deaths. We isolated *Vibrio* in 29% (5/17) of the rectal swab samples from Bogra and in 40% (8/20) of the rectal swab samples from Kishorganj. We found *Vibrio* in 1 out of 8 tap water samples from Bogra and both the tap water samples from Kishorganj. We did not find *Vibrio* in the water samples from central pumps or tube wells. Ground water extracted from deep tube wells was supplied intermittently through interconnected pipes without treatment in both outbreak areas. We found visible leakages in pipelines in Bogra. Though we found no visible leakages, but pipes passed through open sewers in Kishorganj. The rapid onset of severe watery diarrhea in adults and isolation of cholera organisms from their rectal swabs confirmed that the outbreaks were caused by *Vibrio cholerae*. The detection of *Vibrio* in the tap water samples but not from central pumps or tube wells, suggested water contamination in the pipelines. Safe water provision is difficult in municipalities where water supply is intermittent, and where pipes commonly leak; and requires actions outside of the health sector. Collaborative research exploring effectiveness of water purification strategies, including chlorination in areas with intermittent water supply, may identify appropriate approaches for ensuring safe water until improvement of the water and sanitary infrastructure.

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ACCEPTABILITY, FEASIBILITY AND SUSTAINABILITY OF DUAL PIT LATRINES FOR RURAL HOUSEHOLDS IN BANGLADESH

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In Bangladesh, single pit pour-flush latrines that separate human feces away from a child's environment are commonly installed. But latrine water seals are frequently broken by owners to reduce the volume of water used, thus extending the time for pits to fill. They also break the pit liner and allow latrine contents to overflow. Up to 70% of rural households have visible feces on or around latrines. Latrines with two pits, one for current use and a second for subsequent use when the first is filled and composting, may reduce fecal exposure. We investigated an on-going dual pit latrine construction project to identify the acceptability, feasibility, sustainability, perceived benefits and barriers among impoverished rural households. From October 2010 to April 2011 we enrolled households in a communities where BRAC, a non-governmental organization, implemented a project in 2007 providing dual pit latrines for households that met the Government of Bangladesh definition of 'hardcore poor' who shared the cost of transportation and labor. We conducted group discussions (5), interviewed household members (15) and observed their latrine use at four key stages: immediately after installation, upon switching to the second pit when the first was full, during decomposition of the first pit contents, and during emptying and disposing of the first pit contents. None of the households reported latrine overflow or breaking the pit liner or water seals and we observed no visible feces on or around latrines. Participants perceived the main benefit of using the dual pit latrine was when the first pit became full; households immediately started using the back-up pit without having to empty the fresh feces. After approximately one year, households could empty the decomposed pit contents themselves. This saved money and the decomposed excreta of the first pit could be used as manure. Disgust when switching pits was described as a barrier, but did not discourage switching: 8 households successfully completed pit switching at least once. Subsidized dual pit latrines were acceptable to impoverished households in rural Bangladesh and provided safe and effective separation of feces from the environment. The dual pit latrine should be evaluated among other groups on a larger scale.

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MOTIVATING CONTINUED USE OF POINT OF USE WATER TREATMENT IN RURAL BANGLADESH

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Point of use (POU) water treatment can prevent diarrhea, though most interventions fail to achieve continued use in low income countries. In October 2010, we undertook a 13-month pilot POU water treatment intervention with sodium dichloroisocyanurate (NaDCC) tablets in 3 rural communities in Bangladesh. Trained local female community health promoters (CHPs) made 2 household visits and conducted 1 courtyard meeting per month. They encouraged water treatment by appealing to both health benefits and non-health values including convenience, nurture and modernity; addressed barriers; and provided a free supply of NaDCC tablets for daily use. At the last visit, CHPs gave study participants enough NaDCC tablets to last for two months. We assessed barriers to long term POU water treatment uptake and evaluated the effectiveness of the intervention in addressing these barriers during and at the end of pilot intervention activities. We assessed use by testing for residual free chlorine in stored drinking water in study households at the 2nd month (n=129) and 14th month (n=91). We also interviewed mothers of <5 children (n=30) and conducted group discussions (n=6) with both male and female study participants at 14 months. At months 2 and 14, 82% (106/129) and 62% (56/91) of households had detectable free residual chlorine in stored treated water, respectively. Respondents reported that they had become accustomed to the smell, taste and temperature of stored treated water and no longer perceived them as barriers. Respondents reported reduced episodes of illness, especially stomach aches, compared to the previous year, ease of dosing with provided storage vessel and clarity of treated water as factors that motivated continued use. Respondents welcomed regular household visits by CHPs whom they knew as neighbors. They

emphasized that the encouragement provided by the CHPs motivated them to use NaDCC tablets in spite of their initial reaction to stored treated water, their heavy workload, and the reluctance of males to drink treated water. Despite initial concerns with smell, taste and temperature, the majority of study participants continuously treated their water for 13 months and at least one month after active promotion ended. Combining access to effective, easy-to-use water treatment technology with trained, confident and knowledgeable local community health promoters can help improve the uptake of POU water treatment.

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THE NEED FOR POINT OF USE WATER TREATMENTS IN AREAS OF PERI-URBAN POVERTY: CASE STUDY OUTSIDE IQUITOS, PERU

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This study aimed to determine the water collection and home treatment methods in Belen, Iquitos, Peru to elucidate the cause of the high incidence of gastrointestinal diseases in this neighborhood. The results of this study emphasize the importance of point of use water treatment in the home. Belen is a sector of urban poverty on the outskirts of Iquitos, capital of the Loreto region of Peru, where people suffer from gastrointestinal diseases at higher rates than the rest of the city. While many previous studies have highlighted the prevalence of several specific pathogens in this neighborhood, there is little information regarding water-collection methods and treatment in this region. In July 2011, 50 households located in Belen were surveyed using stratified random sampling. Surveys were administered to the head of household in Spanish. In each house, a water sample was collected from the primary drinking water source in a sterile cup with an airtight screw cap then transported to the *Universidad Nacional de Amazonica Peruana* microbiology lab for fecal coliform (FC) testing. The American Public Health Association guidelines were used for FC testing. The overall rate of contamination was 11.1%. Most of the water samples (92.5%) that were negative for FC were untreated in the home, suggesting that treatment methods used by the local water provider are sufficient at the point of treatment. The positive FC sample results most likely represent contamination during the time of storage or use in the home. No sample that had been treated at home had a positive FC test. Therefore, it will be critical to emphasize to residents of Belen the importance of home water treatment before consuming water. It is possible that water is contaminated during storage, so residents should be urged to keep their water storage containers disinfected as well. Iquitos boasts a water plant with treated water, but the amount of chlorine may not be adequate to cover contamination en route or in the home. Although a region may have access to treated water, residents should continue to practice point of use treatment to ensure the safety of their drinking water. Point of use contamination could be a substantial source for fecal contamination and therefore point of use treatment should be encouraged in the homes of communities of peri-urban poverty similar to Belen.

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MEASURING CONTAMINATION OF CHILDREN'S TOYS TO EVALUATE HOUSEHOLD SANITATION IMPROVEMENTS IN RURAL BANGLADESH

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The impact of modest improvements in sanitation facilities and practices on community health are unknown. As one step to better understand the potential contribution of such modest improvements, we evaluated whether different levels of sanitation are associated with environmental contamination, as indicated by fecal contamination of children's toys, in rural Bangladesh. We assigned 100 households to the "clean" category if they had an improved latrine and no visible human feces in the living or adjacent space, or to the "less clean" category if they had an unimproved latrine and visible human feces in living or adjacent space. We distributed two non-porous toy balls to each household, washed each toy in 200 ml of Ringer's solution 3-4 days later, and repeated the process with two new toys. We enumerated fecal coliforms and fecal streptococci in the wash fluid from each toy following standard procedures. Toys from 39 clean households had lower average fecal coliform contamination than toys from 61 less clean households (mean of $\log_{10}$transformed values 2.4 versus 3.2, p= 0.03). Fecal streptococci contamination was not significantly different between clean and less clean households (mean of $\log_{10}$transformed values 4.7 versus 4.8, p = 0.37). There was substantial variability in fecal coliform contamination of two toys in the household at the same time (Coefficient of Variation (CV)=36.5), and toys in the household at two different times (CV=37.6). In rural Bangladesh improved sanitation structures and practices were associated with less environmental contamination. Whether this level of difference in environmental contamination improves child health merits further study. The level of variation of this measure was typical for measures of environmental contamination, such as measures of water quality. Sentinel toy contamination may be a useful objective measure to assess the ability of sanitation interventions to reduce fecal contamination.

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ETHNOGRAPHIC AND DIARRHEA PREVALENCE RESULTING FROM COMMUNITY BASED WATER TREATMENT SYSTEMS: A COMPARISON BETWEEN FINDING IN UGANDA AND HONDURAS

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Using a combination of ethnographic methods, healthcare facility chart reviews, and individual waterborne parasite tests, this paper presents the results of a three year investigation comparing the health impacts of providing water treatment systems for communities in Uganda versus Honduras. The Honduras project provided treated water and flush toilets for an approximated 340,000 people. Improvements in health were documented in Honduras by ethnographic findings, parasite surveys, and medical chart reviews, and were confirmed by local public health officials. In Uganda, no such impact was documented despite provision of access to treated water meeting US, EPA standards and the universal knowledge of waterborne illnesses and their causes within the six communities studied. Ethnographic data and subsequent KAP survey data confirmed accurate local understandings of water and health issues as well as significant gaps in the water safety behavior. A total of 19,420 patient interactions were searched for possible waterborne illnesses within both test and control communities and showed no significant differences in rates of diarrhea and/or dysentery. Random selection of subjects for parasite surveys by

rapid stool tests also showed no significant differences between test and control communities. Likely causes of these findings will be discussed including the probability that exposure to contaminated water in Lake Victoria, animal feces, and open-air food sources contribute to ongoing disease loads within the test communities. Methods developed and implemented for this study represent a significant advance over commonly used survey techniques.

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CLINICAL TOLERABILITY OF ARTESUNATE-AMODIAQUINE VS. COMPARATOR TREATMENTS FOR UNCOMPLICATED FALCIPARUM MALARIA IN SUB-SAHARAN AFRICA, AN INDIVIDUAL PATIENT DATA ANALYSIS

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The wide-spread use of artesunate-amodiaquine (ASAQ) for treating uncomplicated malaria makes it important to gather and analyse information on its tolerability. An individual-patient tolerability analysis was conducted using data from eight randomized controlled clinical trials conducted at 17 sites in nine sub-Saharan countries comparing ASAQ to other antimalarial treatments. All patients who received at least one dose of the study drug were included in the analysis. Differences in adverse event (AE) and treatment emergent adverse event (TEAE - AE which were absent pre-treatment or worsened with treatment) were analysed by Day 28. A total of 6,179 patients were enrolled (74% <5 years of age), of whom 50% (n=3,113) received ASAQ, 20% (n=1,217) another ACT, and 30% (n=1,849) a non-ACT (combination or single-agent) treatment. Overall, 8,542 AEs and 3,943 TEAEs were recorded. The proportion of patients experiencing at least one gastro-intestinal AE on ASAQ was 43% (higher than with artemether-lumefantrine and dihydroartemisinin-piperaquine at two sites only), and was 23% for any other AEs (not different from other treatments). Specifically, the risk of diarrhoea, vomiting, cough and weakness was lower with artemether-lumefantrine; artemether-lumefantrine and dihydroartemisinin-piperaquine carried a higher risk of pruritus, chloroquine-SP of nausea. Parasitological recurrence increased the risk of occurrence of any AE. No other difference was detected. Comparing AE to TEAE in patients who had pre-treatment occurrence and grades of intensity recorded, AEs were significantly more related to the pre-treatment prevalence of the symptom (p=0.001, Fischer test); AEs overestimated TEAEs by a factor ranging from none to 5-fold. The overall incidence of serious AEs (SAEs) with ASAQ was nine per thousand (29/3,113) and a mortality of one per thousand (three deaths, none drug-related) and similar to other treatments. ASAQ was comparatively well-tolerated. Safety information is important, and must be collected and analysed in a standardised way.

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TOWARDS RATIONAL USE OF ANTIBIOTICS FOR SUSPECTED SECONDARY INFECTIONS IN BURULI ULCER PATIENTS

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The emerging neglected disease Buruli ulcer is treated with streptomycin and rifampicin and surgery if necessary. Frequently other antibiotics are used during treatment. Information on prescribing behavior of antibiotics for suspected secondary infections and for prophylactic use was collected together with cultures from ulcers. Of 185 patients that started treatment for Buruli ulcer in different centers in Ghana and Bénin 51 were admitted. Forty of these 51 admitted patients (78%) received at least one course of antibiotics other than streptomycin and rifampicin during their admission. The median number (IQR) of antibiotic courses for admitted patients was 2 (1, 5). Only twelve patients received antibiotics for a suspected secondary infection, all other courses were prescribed for use as prophylaxis during 10 days on average after excision, debridement or skin grafting. Antibiotic regimens varied enormously per indication. Cultures from superficial swabs showed the expected bacteria from a chronic wound, but 13 of the 34 (38%) *S. aureus* showed to be MRSA. A guide for rational antibiotic treatment for suspected secondary infections or prophylaxis is needed. Adherence to the proposed guideline may reduce and tailor on antibiotic use other than streptomycin and rifampicin in Buruli ulcer patients. It may save costs, reduce toxicity and limit development of further antimicrobial resistance. This topic should be included in general protocols on the management of Buruli ulcer.

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THE ASSOCIATION BETWEEN MALARIA PARASITEMIA, ERYTHROCYTE POLYMORPHISMS, MALNUTRITION AND ANAEMIA IN CHILDREN LESS THAN 10 YEARS IN SENEGAL: A CASE CONTROL STUDY

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Malaria and anaemia (Haemoglobin<11 g/dl) remain frequent in sub-Saharan Africa. the ethiology of anaemia is known to be multi-factorial, most studies in malaria endemic areas, have been confined to analysis of possible associations between anaemia and individual factors such as malaria. A case control study involving children aged from 1 to 10 years was conducted to assess some assumed contributors to anaemia in the area of Bonconto Health post in Senegal. Study participants were randomly selected from a list of children who participated in a survey in December 2010. Children aged from 1 to 10 years with haemoglobin level below 11 g/dl represented cases (anaemic children). Control participants were eligible if of same age and their haemoglobin

level was >= 11 g/dl. For each participant, a physical examination was done and anthropometric data collected prior to a biological assessment which included: malaria parasitemia infection, intestinal worm carriage, G6PD deficiency, sickle cell disorders, and alpha-talassemia. Three hundred and fifty two children < 10 years of age were enrolled (176 case and 176 controls). In a logistic regression analysis, anaemia was significantly associated with malaria parasitaemia (OR=5.23, 95%CI [1.1-28.48]), sickle cell disorders (OR=2.89, 95%CI[1,32-6.34]), alpha-thalassaemia (OR=1.82, 95%CI[1.2-3.35]), stunting (OR=3.37, 95%CI [1.93-5.88], age ranged from 2 to 4 years (OR=0.13, 95%CI[0.05-0.31]) and age > 5 years (OR=0.03, 95%CI[0.01-0.08]). No association was found between G6PD deficiency, intestinal worm carriage and children's gender. Malaria parasitaemia, stunting and haemoglobin genetic disorders represented the major causes of anaemia among study participants. Aneamia control in this area could be achieved by developing integrated interventions targeting both malaria and malnutrition.

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ASSESSMENT OF THE ULTRASOUND EXAMINATION AS AN EPIDEMIOLOGICAL TOOL FOR THE SECONDARY AND TERTIARY PREVENTION IN A MALIAN RURAL AREA

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Rural populations have less access to preventive health care and routine medical tests than residents of large cities. Ultrasonography is a non-invasive method that can aid in the diagnosis of a variety of conditions that require medical intervention. To assess the utility of ultrasound as a diagnostic screening test in a rural setting, five ultrasound examination visits were held in the 11 villages of Sabougou health area in Kolokani district (population 10,999 inhabitants in 2010). The village chief provided the examination site in 10 of the study villages and the local health clinic (CSCOM) was used in Sabougou. The motorcycle of the CSCOM was used, as well as a power generator and a portable ultrasound machine equipped with two probes of different frequencies. All volunteers (n=782) underwent a brief medical examination and ultrasound examination of the abdomen and heart performed by two physicians, including a well-trained ultrasonographer. In addition, women older than 15 years of age (n=416) underwent uterine ultrasound, male subjects of all ages (n=272) underwent scrotal ultrasound and all individuals older than 15 years of age (n=588) underwent thyroid ultrasound. Of the 782 subjects examined, 194 (25%) were less than 15 years old, 64 (8%) were pregnant women and 53 (7%) were > 65 years old. The overall prevalence of cardiac valvular calcification was 5% (39/782), and 0.64% (5/782) subjects had evidence of ventricular dilatation. Among the 272 men examined, 22 cases (8.09%) of subclinical hydrocele, 11 cases (4.04%) of hydrocele, 5 cases of testicular cysts (1.84%),3 cases of prostatic adenoma (1.1%) and 1 case of prostatic cancer were identified. Two of the 510 women (0.39%) examined had uterine fibromas and one case of uterine malignancy was detected. Among the 64 pregnant women, one case of fetal demise (1.6%) and one case of extra-uterine pregnancy (1.6%) were detected. One case of multiple abnormalities of the thyroid, heart and testis was also observed. A total of 117 and 28 subjects were referred for further management to the Sabougou community health center and the Kolokani district reference center, respectively. Given these results, ultrasound examination in remote rural areas is a practical and non-invasive method for the identification of individuals requiring referral for medical care in rural Mali and its use should be considered at a regional and national scale.

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EVALUATION OF NEW TECHNOLOGY MULTIPLEX NUCLEIC ACID TESTS FOR EMERGING AND TROPICAL BLOODBORNE PATHOGENS

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Testing of bloodborne pathogens has reduced the risk of transfusion-transmitted infections significantly and use of molecular diagnostic tools has further improved the accuracy of diagnosis. However, with increasing numbers of emerging pathogens that can impact blood safety and the potential for multiple infections in a tropical setting, it is becoming burdensome to conduct separate tests for each agent. Devices that allow simultaneous testing for multiple pathogens (multiplex testing) can potentially streamline blood donation and diagnostic testing. We evaluated two devices, the OpenArray® by Life Technologies Corp. (Carlsbad, CA), and the Resequencing Pathogen Microarray (RPM) by TessArae, LLC (Potomac Falls, VA), for their potential ability to enable multiplex testing in whole blood and plasma samples. The OpenArray® system can perform approximately 3,000 individual real-time polymerase chain reaction tests simultaneously on a microscope slide-size metal wafer. The RPM utilizes an Affymetix© GeneChip© base and a particular arrangement of oligonucleotides. Hybridization to these oligionucleotides leads to sequence identification. We assembled and tested a blood pathogen OpenArray® with primer and probe sets for viruses (HIV-1, HCV, HBV, WNV), parasites (*Trypanosoma cruzi*, *Leishmania*, *Plasmodium*), and Gram negative bacteria. Simultaneous detection of these 4 viruses in plasma specimens and 5 bacterial or parasite species in whole blood specimens was achieved at limits of detection equivalent to individual assays. An RPM was designed and tested with tiles for 22 viruses, 53 prokaryotes and 25 eukaryotes. We correctly identified nucleic acid from 10 pathogens simultaneously. These two multiplex detection devices are highly specific for known bloodborne pathogens. Future testing will reveal whether the sensitivity of these platforms is adequate and whether they are feasible for use in clinical diagnostic and blood donation settings.

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CENTRE-BASED CLINICAL MANAGEMENT OF CYSTIC ECHINOCOCCOSIS

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Cystic echinococcosis (CE) is one of the world's most neglected diseases. The lesions, predominantly in the liver and lungs, develop clinically silently over long periods of time until complications suddenly precipitate. The challenge for health care services, in particular in low-resource settings, is twofold, early detection and treatment of cases and very demanding management of complications of late stage disease. In high-income countries mostly migrants from CE-endemic areas are affected. In this setting CE is not only neglected but also rare and health services are, as a rule, not experienced to diagnose, stage and manage this disease appropriately. A centre-based approach of CE is presented with our interdisciplinary clinical CE unit at Heidelberg University Hospital as an example. Infectious disease / tropical medicine physicians, radiologists, abdominal and thoracic surgeons, gastroenterologists and parasitologists work very closely together to stage patients (ultrasound-based cyst classification) and to tailor currently available mostly expert-opinion based treatment options (medical treatment with albendazole, percutaneous cyst-sterilization techniques, surgery and 'watch and wait') to the needs of the individual patient. This approach can serve as a model for the clinical management of many other NTDs / NIDs in highly mobile global populations.

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TUBERCULOUS MENINGITIS AND RABIES ARE THE MOST COMMON CENTRAL NERVOUS SYSTEM INFECTIONS IN THE NATIONAL REFERRAL HOSPITAL FOR INFECTIOUS DISEASE, THE PHILIPPINES

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Central nervous system (CNS) infections are significant causes of mortality and mobility in low-middle income countries. To improve clinical diagnosis, management and public health intervention, it is essential to clarify the comprehensive picture of CNS infections. However most published studies focused limited pathogens. The objective of this study is to describe the present picture of whole CNS infections in the Philippines. We conducted a hospital-based retrospective descriptive study in San Lazaro Hospital (SLH), the national referral hospital for infectious and tropical diseases in the Philippines. We collected demographic and clinical information of all patients who were admitted with any suspected CNS infection from 1st January 2008 to 30th September 2011. It included all patients who were diagnosed CNS infections as initial and/or final diagnosis and all hospitalized patients who required a lumbar puncture (LP) examination, except for patients in HIV ward. A total of 1,264 patients were analyzed, 937 of them showed CNS infections as final diagnosis. There were more males (62%) and nearly half of the cases (43%) was under 12 years old. Tuberculous meningitis (TBM) and Rabies were the most common CNS infections with 312(27%) cases of TBM and 217(19%) of rabies. This was followed by other bacterial meningitis 169(15%) and viral encephalitis 135(12%). Case fatality rate (CFR) for rabies was 100%; likewise, the CFR for non-rabies CNS infection was also high at 238/703(33.9%). 187(16%) of the patients who were initially diagnosed as CNS infections were confirmed not CNS infection in the final diagnosis. Febrile convulsions and seizures were the most common non-CNS infections. 131(11%) of the patients who were initially not diagnosed as CNS infections were later diagnosed as CNS infection: typhoid fever was the most common misdiagnosis upon admission. LP was performed in 277(22%) cases but its performance was often substantially delayed since many of the patients were critically ill upon admission and none of CSF was positive for bacterial culture. There is ample room for improvement of clinical diagnosis and management of CNS infections.

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DENGUE AND DIARRHEAL DISEASE RISK FACTORS IN RURAL AND SUBURBAN VILLAGES IN THAILAND AND LAOS

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Diarrheal diseases and dengue fever are major global health problems. Household drinking water (DW) storage can be a determinant for both diseases if water is fecally contaminated and the storage containers provide breeding sites for dengue mosquitoes. The aim of this project is to assess health risks associated with household water storage practices by identifying relationships between household water management, contaminated DW, and mosquito production. In 2011 we collected entomological, bacteriological, and socioeconomic data from one rural and one suburban village in northeastern Thailand and southern Laos, respectively. In rural Thailand, almost 100% of the study population use rainwater as DW. In rural Laos 83% use unprotected wells in the dry season and 92% use rainwater in the rainy season. In the suburban settings DW sources are rainwater and bottled water. There was an average of 2.5 DW containers per household. Only 6% of households in rural Thailand and 43% in rural Laos treat their DW. These figures were higher for the suburban areas (Thailand: 65%; Laos: 84%). Water holding containers were found in >93% of the households, of which ~19% were positive for *Aedes aegypti* immatures. The most productive containers were cement tanks in both countries, representing 15-17% of all encountered pupae. The Breteau index (BI) was higher in Thailand than in Laos (140 vs 845, $p<0.01$). In Thailand the BI was higher in the rural area than in the suburban area (147 vs 134, $p<0.01$), whereas in Laos the opposite was observed (112 vs 56; $p<0.01$). In Thailand almost 10% of the *Aedes* positive containers were used for drinking, whereas in Laos as many as 25% were used for drinking. Of the *Aedes* infested DW containers 26% in Thailand and 51% in Laos were also contaminated with *Escherichia coli*. The results suggest an intricate relationship between water contamination and mosquito production in household water storage containers. This relationship and the role of domestic water management practices as risk factors for both dengue and diarrheal disease will be discussed.

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SAFETY AND EFFICACY OF ARTEMETHER-LUMEFANTRINE AGAINST UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA DURING PREGNANCY: A SYSTEMATIC REVIEW

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Plasmodium falciparum malaria during pregnancy, linked to increased morbidity and mortality, must be reduced by preventive measures and effective case management. Although, the World Health Organization (WHO) recommends artemisinin-based combination therapy (ACT) to treat uncomplicated *P. falciparum* malaria during the second and third trimesters of pregnancy, and quinine plus clindamycin during the first trimester, the national policies of many African countries currently recommend quinine throughout pregnancy. Our objective is to analyze available data on the safety and efficacy of artemether-lumefantrine (AL) in pregnancy. English-language search identified 16 publications from 1989 to October 2011 with reports of artemether or AL exposure in pregnancy, including randomized clinical trials, observational studies, and systematic reviews. There were 1,103 reports of AL use in pregnant women: 890 second/third trimester exposures; 212 first trimester exposures; and 1 case where the trimester of exposure was not reported. In the second and third trimesters, AL was not associated with increased adverse pregnancy outcomes compared with quinine or sulphadoxine-pyrimethamine, showed improved tolerability relative to quinine, and its efficacy was non-inferior to quinine. Few reports suggest that the pharmacokinetics of anti-malarial drugs may change in pregnancy, however, the majority of studies reported high cure rates and adequate tolerability. Additional data are required to assess the potential to use AL in the first trimester. These findings reinforce the WHO recommendation to treat uncomplicated *P. falciparum* malaria with quinine plus clindamycin in early pregnancy and ACT in later pregnancy.

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MICROBIAL ETIOLOGY OF TRAVELERS' DIARRHEA: EXPERIENCE OF A TRAVEL CLINIC IN TOKYO

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Travelers' diarrhea (TD) is the most common illness in international travelers visiting developing regions of the world. Published studies provide relatively limited data on the microbial etiology of TD from South and Southeast Asia, which is popular destinations for tourism and business from Japan, compared with that from Africa and Latin America. Travelers who visited at the Travel Clinic of the National Center for Global Health and Medicine, Tokyo, with acute diarrhea (<14 day) that started during or shortly after a stay abroad during December 2009 and March 2012 were eligible for this study. After the participants provided informed consent, clinical data and stool samples were collected. The stool samples were screened by PCR for conventional diarrheagenic bacterial pathogens and cultured by standard methods. Commercially available antigen detection kits for *Giardia*, *Cryptosporidium*, rotavirus, norovirus, and adenovirus were also used. A total of 121 cases were analyzed. The major destinations included Southeast Asia (44%), South Asia (30%), and Africa (13%). Diarrheagenic pathogens were detected in 66% of the TD cases. In 23% of them, multiple pathogens were detected in the stool samples. Enterotoxigenic *Esherichea coli* was the most common pathogen in all the destinations (36%). Enteroaggregative *E. coli* was the second most common pathogen overall (12%) and more frequently detected in the cases who had returned from Southeast Asia. *Campylobacter, Shigella*, and rotavirus followed in this order. Rotavirus was more frequently detected in the cases who had returned from South Asia ($P < 0.05$). Ciprofloxacin resistance in diarrheagenic *E. coli* was rare in all the destinations, but broad-spectrum β-lactam resistance was found in the strains from South Asia. Further investigation focusing on antimicrobial resistance of pathogens of TD is needed.

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DISTRIBUTION OF RUBELLA INFECTIONS IN RWANDA SINCE 2003

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Rubella virus is the causative agent of the disease known more popularly as German measles and is predominantly a childhood disease, endemic throughout the world. Natural infections of Rubella occur only in humans and are generally mild but complications, most commonly polyarthralgia in adult women, do exist. RV infection of women during the first trimester of pregnancy can induce a spectrum of congenital defects in the newborn, known as congenital rubella syndrome (CRS). Since 2003, the National Reference Laboratory of Rwanda has been involved in the surveillance of Rubella infection throughout the country. Cumulative data show that of the 1,778 samples suspected of Rubella, 382 were positively identified by ELISA (21,5%). In Rwanda, geographical data indicates that the Rubella is equally distributed in all provinces of Rwanda with small pockets of infections in Kigali city and Ruhango district, close to the border of Burundi. According to sex and age, infections occur equally in males and females but the majority of infections were in patients older than 5 years old (69,9%).

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ESTABLISHING A TROPICAL MEDICINE TRAINING PROGRAM FOR THE US DEPARTMENT OF DEFENSE (DOD) IN KINTAMPO, GHANA: OVERCOMING CHALLENGES

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Endemic diseases remain key concerns when deploying US forces. A critical challenge for us is to maintain a solid fund of knowledge in tropical medicine. DoD has courses that provide such training. We discuss challenges of developing a Ghana field site as part of an advanced course, Military Tropical Medicine-Field. Logistics: The annual 2 week event launched in August, 2008, at War Memorial Hospital in Navrongo. This provided a remote setting. However, an 18 hour drive from Accra was problematic. The site was moved to Kintampo, 7 hours from Accra. The right partner: We initially partnered with the Ghana Army at the 37 military hospital. This lacked the disease burden for the course objectives. The Kintampo site had a District Hospital and a Clinical Research Center (CRC). Our partnership began at CRC. It had a strong lab program. However, the students already complete a lab curriculum during pre-requisite courses. Direct patient contact embedded in Ghanaian conduct of clinical care and public health was critical. A stronger relationship with the hospital resulted. An exchange program: The Ghana team is limited to ten physicians. While team backgrounds vary, the course faculty focuses on Preventive Medicine and Infectious Diseases. The hospital provides additional focus in Surgery, Pediatrics, and through educational collaborations, Emergency Medicine. Neither the US students nor the faculty practice independently. Through active shadowing, Ghanaian led care delivery teaches the team. Differences in care and differential diagnoses are discussed. Nurturing the relationship: Continuity is important and we maintain contact with our hosts during the 11 month hiatus. Each year we execute a planning visit. We also support our Ghanaian partners. For instance, we nominated our host physicians to activities such as the University of Florida epidemiology course. Humanitarian Assistance: In 2008, we provided US led direct care in a village. We have discontinued this activity because of the risk of undermining host medical infrastructure and difficulties following patients. Financial resources restricted by law instead are applied to resource our hosts with locally procured consumables based upon their and our needs assessments as well as the course's curricular objectives. This durable and multi-faceted relationship has allowed us in the last four years to optimize this episodic learning environment on the ground.

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DECREASING OCCURRENCE OF TROPIC NEUROINFECTIONS: CEREBRAL MALARIA, MENINGOCOCCAL MENINGITIS AND SLEEPING SICKIES IN SOUTH SUDANESE RURAL HOSPITALS

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With advantages of AIDS treatment, 20 years ago some of tropical neuroinfections (e.g. cryptococcal meningitis, tuberculosis meningitis, CNS toxoplasmosis or CMV encephalitis) showed increasing incidence. However, with the era of HAART they again become to be rare. Also severe malaria, manifesting as cerebral malaria with hypoglycemia acidosis and severe anemia, is less frequent after the introduction of intermittent preventive treatment (IPT) and artemisinin in early treatment. In this study, two rural hospitals - Mapuordit in Yirol country and Gordim in Aweil country (both with 5-20 beds) - were compared. Totally, 18 027 patients were treated in Mapuordit and 9358 in Gordim. Most patients (90%) were treated on outpatients' basis, the rest as inpatients. Both hospitals offered two doctors with surgical and medical qualification and mobile team from Italy is coming for elective surgeons two times a year. Occurrence of severe malaria in Mapuordit (close to Nile River) cases was more common in Gordim. Trends in malaria occurrence correlated with dry and rainy season. Diarrheal diseases showed slight increase in 2011. Pneumonia represented about 20-25% of all respiratory tract infections. Skin and soft tissue infections (SSTI) were fifth common infection after RTI, diarrheal diseases, malaria and sexually transmitted diseases with urinary tract infections showed stable trend and not significant difference between Mapuordit (40-50 a month in average) and Gordim (33-40 per month). Only 1 case of tetanus occurred in both hospitals during observational period. Diphtheria was also sporadic, as well as meningococcal meningitis, tuberculosis and leprosy appeared in 442 patients in Mapuordit and showed a relatively stable trend. HIV was only rarely detected in Mapuordit however only surgical patients were tested as a part of the hospital screening. Commonest ID of both south-Sudanese hospital was malaria and RTI, followed by diarrhea; however, these hospitals are located in different climatic and vegetation areas (close to Nile versus Savannah - Satel type vegetation). Sporadic cases of cholera, meningococcal meningitis and tetanus were observed in Gordim and outbreak of meningitis in 2009 in Mapuordit as well. Occurrence of tuberculosis and leprosy showed a stable trend in both hospitals between periods (2009-2011). HIV was exceptional probably due to isolation and civil war independence declaration in 2011.

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HEALING OF CUTANEOUS LARVA MIGRANS AFTER A SINGLE DOSE OF IVERMECTIN IS ACCOMPANIED BY CHANGES IN CYTOKINE PATTERNS IN PERIPHERAL BLOOD

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Cutaneous larva migrans (CLM) is a neglected tropical skin disease caused by the migration of animal hookworm larvae in the epidermis. The disease is common in resource-poor communities in developing countries. Patients with CLM were identified through active case finding in two resource-poor communities in Manaus, Brazil. Patients were diagnosed clinically, and severity of the disease was assessed using a semi-quantitative severity score. Clinical pathology was assessed and hematological and immunological investigations were performed before, and two and four weeks after treatment with ivermectin (200µg/kg). Leucocytes and eosinophils were counted and total serum IgE was determined. The concentration of IL-4, IL-5, IL-10, IFN-γ, TNF-α and TGF-β was determined in serum using commercially available ELISA kits. 92 patients were included in the study: 69.6% were male and 30.4% were female. Median age was 9.5 years (IQR 5-44). At baseline, 93.4% of all patients complaint about severe pruritus and 73.6% about insomnia. The median severity score was 4 points (IQR 3-6). 87.8% of the patients had eosinophilia. Patients with CLM had significant higher concentrations of IgE, eosinophils, IL-4, Il-5 and IL-10 in serum than age- and sex- matched controls living in the same community. Four weeks after treatment, clinical pathology and eosinophila decreased significantly. While the serum concentration of IL-4, IL-5 and IL -10 decreased, the concentration of IFN-γ increased significantly. It is concluded that in an impoverished community CLM is associated with considerable morbidity. After treatment with ivermectin, clinical pathology, eosinophilia and cytokine patterns normalize rapidly.

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SPECTRUM OF TROPICAL NEUROINFECTIONS AND OTHER INFECTIOUS DISEASES IN OUR LADY OF FATIMA HOSPITAL, GORDIM, SOUTH SUDAN, BEFORE AND AFTER DECLARATION OF INDEPENDENCE

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For 10 years, the area of South Sudan was under control of Sudan´s People Liberation Army (SPLA) and medical serviced were served by Médicins Sans Forntiéres (MSF) and other humanitarian organizations after the declaration of independence in May 2011, when country was completely opened for foreign travel. Aim of this study is to compare the spectrum of tropical infectious diseases (ID) before and after independence declaration (2010 vs. 2011) in rural hospital in Gordim, South Sudan. In 2010, together 5097 outpatients were compared to 3612 outpatients treated in 2011. Diagnostic of malaria, geohelmints and tuberculosis (TB) has been performed by microscopy, as rapid diagnostic tests (RDT) were not available until spring 2012. HIV was not observed in none of outpatient department (OPD) patients. Trends in TB were stable with 30 - 60 new cases per month. However, trends in malaria correlated with rainy season, with maximum of 329 and 367 cases in August and September, respectively. Fortunately, complicated malaria (cerebral malaria, renal failure) were extremely rare, probably because of early treatment with artesunate or artesunate/lumefantrin. Second commonest diseases were respiratory tract infections (RTI) with mostly stable occurrence of 260 - 293 cases per month, followed by diarrhea and sexually transmitted diseases (STD). Only 1 case of tetanus occurred during 2010 - 2011. There was observed only 1 case of cerebral malaria, 8 cases of meningococcal meningitis and 1 case of sleeping sickness in period of 2010 - 2011, which is very low in comparison with 32 cases of cerebral malaria, 119 cases of meningococcal meningitis and 9 cases of sleeping sickness in period of 2005 - 2006. We can conclude that in Gordim there was no significant difference in tropical ID incidence before and after South Sudan has been completely opened for foreign travellers. Also, occurrence of tropical neuroinfections, such as sleeping sickness, cerebral malaria and meningococcal meningitis, was sporadic.

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EPIDEMIOLOGY OF FEBRILE ILLNESSES AMONG INFANTS: A CASE CONTROL STUDY IN KINTAMPO NORTH AND SOUTH DISTRICTS

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Information on the burden and risk factors of febrile illnesses in infancy is scarce. Young infants are relatively protected against infant illnesses during the first six months of life due to the presence of maternal antibodies and foetal haemoglobin, and have received relatively little attention with respect to research and treatment guidelines. To add to the limited data available, this study therefore sought to determine the predisposing factors to febrile illnesses among infants (0-11months). A case control study was conducted in Kintampo North Municipality and South District. We randomly selected 230 cases and 454 controls from infants with and without infant febrile illnesses and were participants of an ongoing study. Standard questionnaires were administered by blinded interviewers to randomly selected cases and controls. Variables compared in both groups included birth weight, breastfeeding practices, immunization status, household background characteristics and socio-economic status of mothers. Data collected was entered on Microsoft Access and analysed using STATA Version 11.Results of the study showed that malaria was the most prevalent febrile illness. Analysis showed that 70% of febrile cases were above 6 months of age, whilst 91.85% were exclusively breastfed. A significant difference was shown between cases and controls in terms of age and breastfeeding status. Apparent similarity was shown between cases and controls with respect to birth weight, household background characteristics, immunization status, ITN use and socio-economic levels of mothers. The study showed that infants above 6 months and those not exclusively breastfed are more likely to develop febrile illnesses. Information on the epidemiology of febrile illnesses among infants will be essential for designing and interpreting results of clinical trials of drugs, vaccines and other interventions for this vulnerable group.

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PROVIDING HIV EDUCATION TO HEALTH CARE PROVIDERS IN COCHABAMBA, BOLIVIA IN EXCHANGE FOR AMERICAN STUDENT ROTATION AT THE MAIN LOCAL PUBLIC HOSPITAL

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American physicians in training seldom experience the florid variety of infectious diseases found in developing countries and tropical areas. The management of patients is also different in limited resource settings. The number of reported cases of HIV in Bolivia is growing exponentially, but the number of HIV providers is not. Hospital Clinico Viedma is a public hospital in the heart of South America that serves the most underprivileged population in the city. It also is a referral center for the surrounding tropical areas. In February, 2012, the Division of Infectious Diseases at the University of Massachusetts coordinated an elective for UMass Medical Students in Cochabamba Bolivia. The elective consisted of 3 medical students, 4 faculty members and a Bolivian Infectious Diseases fellow who served as the course coordinator/director. A major goal of the interchange between the two institutions is to provide basic HIV education for Bolivian health care practitioners in the Cochabamba area in Spanish, the main native language. We accomplished this by teaching an intensive course, involving 10 hours of didactics and 5 hours of case presentation. This course was judged to be outstanding by the participants, although they felt that the course should be expanded to teach the care of pediatric patients with HIV. This year we are expanding our course to pediatrics and live state of the art recording. A secondary benefit of expanding our HIV course will be that UMass faculty members will be on site to precept UMass students on elective in Cochabamba in the diagnosis and management of infectious illnesses not commonly seen in the US setting. This DVD will then be available for distribution to additional health care providers in Bolivia and other Spanish speaking countries.

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THE HEALTH AND DEMOGRAPHIC SURVEILLANCE SYSTEM IN RURAL WESTERN KENYA: RELEVANCE TO PUBLIC HEALTH AND RESEARCH ENDEAVORS

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The Health Demographic Surveillance System (HDSS) set up in Kisumu West district, rural western Kenya, is designed to track on a bi-annually basis, evolving health status, demographics and health threats within the catchment area. The program has GPS-located every dwelling unit that exists within the catchment area and has conducted baseline population and housing survey that is monitored through regular surveys. The primary goal for the program is to provide an exhaustive health and demographic data set throughout the catchment area that would be of great interest to potential research collaborators and the local ministry of Health. This paper examines the contributions made by the program towards advancement of public health and research agenda in the catchment area. The Kisumu West HDSS has provided the first steps in developing the linkage of extensive demographic and health data that is tracked over time to patient health care records, beginning with PEPFAR subjects and later expanding to all patients in the study area. The linkage will greatly aid in subject/patient tracking and linking of disease and patient to specific locations with a view of designing targeted interventions. During a recent polio outbreak in western Kenya, the Kisumu West HDSS provided information towards the successful implementation of the immunization campaign. The information included baseline population figures for the target population and village maps to aide movement of the MOH staff in the field. The KWHDSS provides an ideal research platform for clinical and epidemiological studies. Specific examples of how the KWHDSS supports these studies-including the Phase III Malaria vaccine trial will be provided to showcase its relevance to research endeavors. In conclusion, the KWHDSS continues to provide a central analytical framework for work on clinical trials, disease surveillance and public health intervention in the Kisumu West District. The longitudinal nature of the KWHDSS allows better matching of volunteers for clinical trials such as those involving post-marketing surveillance and studies assessing the impact of other health care interventions.

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BEYOND SIMPLE PREVALENCE: ENHANCED DISCRIMINATION OF INFECTIOUS DISEASE-RELATED DATA PATTERNS BASED ON THREE-DIMENSIONAL ANALYSIS

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One important challenge in infectious disease research is to reduce the rate of information loss and/or errors associated with data analysis, including those generated by prevalence-based analyses. To develop and evaluate an alternative approach that addresses these issues, leukocyte

data collected from humans, birds, and bovines affected by different pathogens were assessed with two approaches: (i) non-structured indicators, such as the neutrophil percent, which were determined with bi-dimensional plots and considered the overall (population) disease prevalence, and (ii) structured indicators (indices designed to generate a single line of observations), which were explored with three-dimensional (3D) plots and considered subset-specific prevalence. These approaches revealed that population-based prevalence analyses did not distinguish the leukocyte profiles of disease-negative (D-) and disease-positive (D+) subsets. In contrast, structured indicators assessed with 3D plots revealed patterns which, when used to partition the data, enhanced discrimination of infection: (1) non-overlapping D- and D+ subsets were generated, (2) observations suspected to be false were detected, and 3) in humans infected with malaria, four disease classes were distinguished. Results presented here demonstrate that patterns previously unrecognized in D+ and D- individuals can be identified with structured, 3D analysis, leading to more informative, subset-specific prevalence estimates.

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METRICS OF SUCCESS FOR SOCIAL DETERMINANTS OF HEALTH AND TROPICAL DISEASES

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Numerous measurement problems emerge when considering neglected tropical diseases (NTDs) on their own, and especially in the social determinants of health (SDH) framework. Burden of NTDs is miscalculated because of underestimation of mortality, long-term sequelae, effects on fertility and on pregnancy, cross-generational effects, and synergies of multiple morbidities. In addition, burden-of-disease methodology specifically abstracts from socio-economic context. Disability-Adjusted Life Years (DALYs) attempt to aggregate the effects of every disease on mortality and morbidity, based on prevalence and specific effects of each disease. DALYs were derived so that a life has the same value, and a disease has the same burden, regardless of place of residence, occupation, or income. The SDH framework embodies a different principle of fairness that requires society to prioritize problems of poor and marginalized people. In the SDH approach, it is necessary to allocate investment disproportionately to diseases of poverty and also to the structural determinants that promote poor health in poor populations. Invisibility of socially excluded populations and their health concerns is another methodological challenge. National and subnational averages can show important progress in achieving disease-reduction goals, while obscuring the persistence of NTDs and the concentration of multiple NTDs in family and community clusters. Global campaigns, including the Millennium Development Goals, state targets as national and global averages. Reliance on them as sole indicators of progress in disease reduction reinforces invisibility of persistent clusters afflicted with multiple morbidities of diseases of poverty, even while national statistics improve. 'Elimination as a public health problem' is a term that definitionally could be at odds with the spirit and practice of reducing health inequities. Continued existence of even low levels of impoverishing and often stigmatizing diseases is evidence of persistent inequities.

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RECLAIMING THE 'BETTER HEALTH FOR ALL' MANDATE: A CASE FOR INTEGRATING GLOBAL HIV PROGRAMMING WITH COMPREHENSIVE PRIMARY HEALTH CARE SYSTEMS IN SUB-SAHARAN AFRICA

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Twelve years into the twenty-first century, our world is still grappling with an HIV/AIDS epidemic that has placed great strain on global human and material resources and compounded human suffering; especially in sub-Saharan Africa. While the discovery and improved access to antiretroviral therapy and allied treatment has significantly reduced mortality and morbidity from this disease, it is still positioned to continue to garner prime attention in health discourse and in the allocation of global resources. However, competing health demands in developing countries such as the rising threat of non-communicable diseases and often-neglected communicable diseases amidst a slowly recovering global financial economy are timely prompts for a careful reconsideration of the prevailing approach to HIV funding and programming which has so far privileged this disease to the detriment of overall health. At this critical juncture, a re-evaluation is invaluable if we are to make the smart health investment decisions that would protect better health in the future. I argue that a departure from the current vertical nature of HIV programming is needed to curb its detraction from the development of effective health systems that are sensitive to the totality of local health realities and needs in sub-Saharan Africa. Using support from relevant literature, I trace the evolution of this vertical handling of HIV, its origins in the selective health care model that eclipsed the Alma Ata affirmation and its deleterious effects on health systems; present policy options and recommendations in making the case for an integration of HIV programming with comprehensive primary health care and discuss some of the few available cases that have pursued integration in various forms. Despite the litany of practical difficulties that may dissuaded a global adaptation of such integration, this step is vital if developing countries are to achieve sustainable, efficient and locally owned comprehensive health systems capable of safeguarding better overall health.

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COMBINING HIV/AIDS AND MALARIA INDICATOR SURVEYS IN TANZANIA TO LEVERAGE EXPERTISE AND MAXIMIZE EFFICIENCY IN LARGE HOUSEHOLD SURVEYS

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Development assistance to Tanzania for HIV/AIDS and malaria ($2.3 billion in 2005-10) has permitted intensive scale-up of multiple interventions. Separate, labor-intensive, nationally-representative household surveys are a cornerstone of monitoring and evaluation (M&E) for National AIDS and Malaria Control Programs. In 2007/8 and 2011/12, the National Bureau of Statistics (NBS) and Zanzibar's Office of Chief Government Statistician (OCGS) succeeded in meeting the needs of multiple stakeholders by creating a combined Tanzania HIV/AIDS and Malaria Indicator Survey (THMIS). The THMIS used a two-stage probability sample design implemented by NBS/OCGS, overseen by the Tanzania Commission for AIDS, with technical guidance from Mainland and Zanzibar malaria and AIDS control programs, and MEASURE DHS. Demographic data and HIV/AIDS and malaria knowledge and attitudes, risk behaviors, and intervention use were collected from adults aged 15-49 years. Dried blood spots for HIV testing of adults and malaria rapid tests, thick blood films, and hemoglobin measurement for children were prepared from

fingerstick capillary blood specimens. In December 2011, data collection for the 2011/12 THMIS was initiated in over 9,700 households, with approximately 10,800 women and 8,000 men expected to be interviewed and tested for HIV and 7,500 children tested for malaria and anemia by April 2012. The 2007/8 HIV and malaria prevalence (5.7% and 17.7%, respectively) will be compared to 2011/12 estimates. The THMIS required four months to complete data collection compared to three to four months per each stand-alone survey. In-country costs for 2011/12 THMIS ($2.1 million vs. $900,000 for 2003/4 AIDS survey) were shared by two U.S. Government initiatives (74%), Government of Tanzania, and others. Careful coordination and planning by multiple stakeholders from HIV/AIDS and malaria control produced a single, mutually appealing, nationally representative household survey. This efficiency helps conserve resources needed to document progress toward Millennium Development Goals.

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EVALUATIONS OF HEALTH RESEARCH CAPACITY DEVELOPMENT: A REVIEW OF THE EVIDENCE

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Local research and innovation capacity is essential to improving health outcomes prompting significant investment in strengthening health research capacity (HRCS) in low and middle income countries LMICs. Although funding agencies need to show value for money and implementers want to demonstrate HRCS impact, empirical evaluation evidence on HRCS seems scarce. We conducted a scoping review of published evaluations of HRCS to learn lessons about how to assess its effectiveness and impact especially in the longer term. We searched electronic bibliographic databases, reference lists of relevant articles, reports of funding agencies, and websites, and consulted 'experts' to identify relevant publications using search terms covering training, mentorship, collaborations, partnerships and networks. We assessed the quality of these evaluations using an instrument developed for reviews of community interventions, and synthesized information about the types and design of the evaluation, and the measurement tools and indicators that were used. We identified 593 publications from health, education and management literature that focused on evaluating the development of health research capacity. 31 were primary studies; only 4 (0.7%) were from LMICs; Ghana (2), Vietnam and Pakistan and the quality of these four studies was variable. None used a comparator group; two were retrospective and two used validated tools. All four studies specified objectives and outcome measures, and stressed the importance of engaging senior managers in developing research capacity. Most provided descriptive analyses including both qualitative and quantitative results. HRCS literature is dominated by recounting of programs and experiences with little published evaluation. A much more substantial evidence base on HRCS interventions reported in peer-reviewed publications is needed before we can develop robust evaluations of impact and value for money of investments in HRCS.

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STRENGTHENING RESEARCH CAPACITY WITHIN A GHANAIAN TEACHING HOSPITAL: TEN YEAR PROSPECTIVE STUDY

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A strong research culture within teaching hospitals, supported by robust research infrastructure and the ability to demand and utilise research, is essential to promote evidence-based practice and improve health outcomes. For 10 years senior managers and academics at Komfo Anokye Teaching Hospital (KATH), Ghana have been strengthening the hospital's research systems and creating a critical mass of research expertise among mid-career health professionals (eg. doctors, nurses, managers, ancillary cadres). The research capacity strengthening programme was designed prospectively using a rigorous implementation research approach for designing and monitoring complex interventions. We adapted a published framework for institutional change and used this to design and monitor the programme in collaboration with key stakeholders. The framework enabled us to use mixed methods flexibly and systematically to plan, regularly review and adapt the programme, to identify and prioritise gaps in KATH's research systems and infrastructure, and to derive and use indicators to monitor progress in closing the gaps. One component of the programme was an innovative 1 year, part-time Professional Diploma course (UK award) which taught ~20 students/year to undertake a research project important to their department. Our published course evaluation demonstrated graduates were competent and confident to design and conduct research. Through the programme KATH now has a dedicated research support office and administrators, a biostatistics unit and better successes with exams, grants and publications. By 2007 the Diploma course was managed and taught entirely by Ghanaian faculty (KNUST) and it is sustainable through locally generated funds. KATH/university faculty have extended the course to other sites in Ghana and Zimbabwe (2009-12). There has been some impact on clinical practice (eg. birth injury prevention programme; reduced needlestick injuries). Next priorities are to strengthen systems for utilisation of research results and to consolidate departmental research incentives.

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STRENGTHENING PATIENT-CENTERED COMMUNICATION THROUGH WORKSHOPS AND SELF-REFLECTION: A CLUSTER RANDOMIZED TRIAL AT PUBLIC HEALTH CENTERS IN UGANDA

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The quality of health care at African health facilities is often reported to be poor, linked to low patient attendance and poor health outcomes. In western settings, patient centred approaches, focused on communication between health workers and care seekers, have been advocated to improve quality of services. Such approaches have received little attention in Africa. Rigorous evaluations are required to inform best practice for improving quality of care in resource limited settings. A cluster randomised trial of the PRIME intervention to enhance quality of services is underway at health facilities in rural Uganda. One component of the intervention is a Patient Centred Services package, intended to improve communication with health care seekers, increase attendance at health facilities and improve overall population health indicators. This paper presents the first step in this hypothesised mechanism of change: the

impact of the intervention on health worker communication. We assessed communication between health workers and care seekers at baseline and immediately after the implementation of the intervention at 20 health facilities randomly assigned to intervention or standard care. A total of 26 health care workers and 213 health care seekers participated. Consultations were recorded and rated using the Measurement of Patient Centred Communication method and care seekers were interviewed on exit to provide their assessment of the quality of communication. Patient-centred communication was rated 10% higher ($p<0.008$) by care seekers consulting with health workers who had recently participated in the PRIME intervention compared with those in the standard care arm. A per protocol analysis suggests this increase may be plausibly attributed to the Patient Centred Services component of the intervention. Improvements to quality of care in resource limited settings may be achieved by approaches that reorient services towards patients.

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INSCALE CLUSTER RANDOMIZED TRIAL EVALUATING THE EFFECT OF INNOVATIVE MOTIVATION AND SUPERVISION APPROACHES ON COMMUNITY HEALTH WORKER PERFORMANCE AND RETENTION IN UGANDA AND MOZAMBIQUE: INTERVENTION DESIGN

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If properly trained, equipped and utilized, community health workers (CHWs) delivering integrated community case management (ICCM) for children with diarrhea, pneumonia and malaria can potentially reduce deaths from these infections by 60%. To achieve this outcome it is essential to maintain CHW performance and retention. The inSCALE project aims to increase sustainable coverage of ICCM in Uganda and Mozambique by designing and evaluating innovations for increased CHW supervision and motivation. A combination of participatory research methods were used to identify program gaps, best practices and potential interventions. Quantitative baseline surveys with household members, CHWs and health facility staff were conducted to establish key outcomes and to inform the randomization process. Following extensive formative research and national stakeholder consultations, two interventions were developed in Uganda and one in Mozambique. In Uganda approximately 3500 CHWs in 39 clusters were randomized into a mobile health (mHealth) arm, a community engagement arm, and a control arm. In Mozambique 300 CHWs in 12 clusters will be randomized into a mHealth arm and a control arm. The mHealth interventions in Uganda and Mozambique encompass three main activities: 1) closed user groups to enable free two-way communication between CHWs and their supervisors; 2) weekly ICCM data submission using phones with automated motivational feedback, SMS to supervisors flagging problems for target supervision, and summary ICCM statistics made accessible online to district statisticians; and 3) monthly motivational and constructive SMS to CHWs. The community engagement arm in Uganda will establish health clubs which seek to improve child health and identify health challenges through a community led model with the CHW as its focal point, potentially resulting in 1) improved status and standing of CHWs as key health assets; 2) increased demand for CHW services, and 3) communication to CHWs and other village members that CHW work is important, of value and appreciated. In both countries process evaluation will be conducted and endline surveys will establish impact after 12 months. Main outcomes will be the proportion of sick children appropriately treated, CHW performance and motivation, and cost effectiveness of interventions.

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EVALUATION OF LOW-COST OPEN-SOURCE MHEALTH TOOLS TO SUPPORT A LONGITUDINAL PEDIATRIC DENGUE AND INFLUENZA COHORT STUDY IN NICARAGUA: IMPROVING QUANTITY, QUALITY, TRACEABILITY AND TIMELINESS OF DATA COLLECTION AND MANAGEMENT

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Dengue and influenza are major problems worldwide. The Pediatric Dengue Cohort Study began in 2004 as a collaboration between the University of California, Berkeley, the NGO Sustainable Sciences Institute, and the Ministry of Health in Managua, Nicaragua, to study the natural history and transmission of dengue in children in a community setting in a developing country. In 2007, the Nicaraguan Influenza Cohort Study was added to study the burden and seasonality of pediatric influenza. These studies provide critical epidemiology and transmission data to support detection and prevention approaches around the world, and clinical data and biological samples are used to study viral and immunological determinants of protection and pathogenesis and for development of novel diagnostic assays and algorithms. Currently, ~3700 children aged 0-14 receive medical care through the studies, and data from all clinical visits are systematically recorded. Participants with suspected dengue, influenza or undifferentiated fever are tested by serological, virological, and molecular biological assays, and yearly blood samples are analyzed to detect inapparent infections. To facilitate the logistics and operations, a set of information technologies have been implemented by the study team since 2004. These eHealth tools - electronic medical records, patient and sample tracking systems using barcode and fingerprint IDs, and field logistics support tools for household visits - help to maintain quality control and facilitate compliance with established Good Clinical Practice (GCP) and Good Laboratory Practice (GLP) standards. In the 2012 annual blood collection conducted in the study Health Center and through household visits, a mixed methods approach was used to assess the impact of a new mobile data collection and management technology package using low-cost Android tablets and cell phones with the free open-source software ODK Collect and OpenMRS. Specific advantages in data entry/processing time, accuracy and accessibility, user experience, and cost savings were observed compared to paper and PDA-based tools. Results were shared with the Ministry of Health, along with lessons learned about implementation, for potential scale-up for routine data collection needs in the national public health system.

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PREVALENCE OF PURCHASE OF ANTIBIOTICS WITHOUT PRESCRIPTION IN CHILDREN UNDER FIVE IN PRIVATE PHARMACIES CLOSE TO PRIMARY CARE CENTERS IN PERI-URBAN AREAS OF LIMA, PERU

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The overuse of antibiotics is associated with the increase of resistant pathogens. In developing countries, antibiotics are commonly purchased

at private pharmacies, which are important suppliers of health tips and low-cost medicines. The objective of this study was to determine the prevalence of purchase of antibiotics without a prescription for use in children under 5 years in private pharmacies of peri-urban areas of Lima, Peru. A survey was applied in adults who bought an antibiotic for a child up to five years in a private pharmacy close to a health center in a peri-urban area of Lima, Peru. 287 of the surveyed bought an antibiotic. The prevalence of purchase without prescription was 13.2%. From these 1.7% were due to self-medication and 11.5% were due to indication of the pharmacist. The diseases that were most often associated with the use of antibiotics were 45.8 common cold (45.8%) and acute diarrhea (18.5%) and bronchospasm (18.5%). Diseases that were mostly associated with use of antibiotics without prescription was common cold (50%) and watery diarrhea (28.9%). An overuse of antibiotics in children less than 5 years exists in this setting, especially in diagnoses as watery diarrhea, common cold and bronchospasm, mainly due to medical prescription. Self-medication was found in a very low percentage as well as pharmacy personnel recommendation. Training of medical personnel should be prioritized and legislative measures in relation to the purchase of prescription antibiotics should also be strengthened.

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BUILDING CAPACITY FOR CONDUCTING CLINICAL TRIALS IN VIETNAM

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Vietnam has seen the emergence of new diseases such as SARS and highly pathogenic avian influenza. Other infectious diseases are endemic. Many parties, including the Vietnamese Ministry of Health (MOH) have a strong interest to conduct clinical trials in Vietnam. The MOH is faced with an urgent challenge to develop a system of oversight that follows the Principles of Good Clinical Practice (GCP), which protects the rights and well-being of human subjects. The MOH also recognizes a need to develop the capacity of local research institutions and their personnel to conduct quality research. With the cooperation of FHI 360 and other partners, MOH has made several interventions to improve capacity to conduct clinical trials in Vietnam. As part of the Southeast Asia Influenza Clinical Research Network (SEA ICRN), a new role titled Clinical Trial Support Specialist was developed within FHI 360. Local health professionals were trained in clinical trial regulatory, ethics and operations processes and then provided formal as well as side by side training to study staff of the local hospitals. Existing health system structure and operation were challenges. In a separate but complimentary effort, MOH partnered with FHI 360 to host a series of workshops which included government regulators, and other stakeholders. Open discussions among the groups revealed and prioritized gaps in ethics knowledge, systems, and infrastructure, from which an MOH strategy to build capacity developed. In 2008, MOH issued a GCP document for Vietnam and a regulation to define and operate an Independent Ethics Committee (IEC). The MOH committed to developing an independent ethics system consistent with international standards. Further workshops hosted by the MOH built capacity within the ministry for ethics review. In conclusion, theoversight of clinical trials in Vietnam and the related capacity of Vietnamese institutions have shown significant improvement since 2005. This has been enabled through a coordinated and strategic approach by the MOH and included partnerships with several international institutions. For next steps in the emerging model the MOH will need continuing partnerships that provide technical assistance, monitoring, and support in order to continue this growth.

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REDUCING HEALTH AND HEALTH SERVICE DISPARITIES IN AN ETHNICALLY DIVERSE, HIGH MIGRATION AREA ON THE THAI-MYANMAR BORDER

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Trans-border migration is increasing rapidly worldwide and already involves almost one-quarter billion people. Ethnic diversity among migrants and between migrants and national majority populations into which they move, plus legal eligibility for residence and access to services pose numerous problems for providing health services and control of transmissible diseases. Transborder migration to Thailand, mostly from Myanmar, now accounts for at least 3 million people from many different ethno-linguistic backgrounds. Transborder migration is expected to increase markedly in 2015 following the opening of borders between ASEAN countries. Migrants to Thailand have significantly higher prevalence of malaria, TB and probably HIV than non-migrant residents. PHPT's Access to Care Project to date has surveyed 998 women and men from Chinese, Hmong and Lahu minorities and from the ethnic Northern Thai majority. Survey data show statistically highly significant differences between different ethnic groups, between minorities and the ethnic majority in socioeconomic characteristics (e.g., income, household possessions, education, Thai language ability, health insurance), and between migrants and non-migrant members of the same ethnic group living in the same communities, with respect to: health information, (e.g., knowledge of HIV transmission, prevention, diagnosis and treatment); use of health services (antenatal care, HIV counseling and testing); and reported constraints to use of health services (e.g., service delays, transportation, direct and indirect costs, lack of knowledge of health and health services, language). Analysis of hospital records allow analysis of differences in delays and interruptions in services and severity of illness associated with ethnicity, location and migration status. Effects of interventions (e.g., to date, health education) tailored by results from surveys (e.g., ethnicity, education, Thai language ability, knowledge of health and health services, migration status) are evaluated by before and after assessment.

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KNOWLEDGE, ATTITUDE AND PRACTICES OF HEALTH CARE WORKERS TOWARDS MALARIA CASE MANAGEMENT IN CHANGING MALARIA TRANSMISSION IN NAMIBIA

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Malaria cases in the last 7 years are on the decline in Namibia due to interventions in malaria control implemented by the Ministry of health and Social Services (MoHSS). There is a shift from control of malaria to its elimination; hence it is necessary for interventions to reflect this shift. Early and proper malaria diagnosis and case management are of paramount importance in reducing the parasite reservoir for elimination of the disease. The knowledge and perceptions of Health Care Workers (HCWs) regarding the prevalence of malaria, its diagnosis and treatment were investigated to provide a basis for aligning the training of health care workers to the objective of elimination of malaria. Three malaria endemic regions of Namibia namely Omusati, Caprivi and Kavango representing different malaria risk strata as well as cultural differences and practices within the country were selected. Six Focus Group Discussions (FGDs) and 7 Key informants Interviews (KI) were conducted. FGDs consisting of 6-10 participants were conducted using semi-structured questions to collect data. Three FGDs were conducted in Caprivi region (Katima Mulilo), 2 in Kavango region (Andara) and 1 in Omusati (Onesi). Each group was primarily composed of registered and enrolled nurses from rural clinics, health centres and regional hospitals. Staff members who were managers

were excluded from the FGDs but were still included in the study and interviewed as key informants. There was a general perception that less malaria cases were presented with 90 % of Health Care workers having knowledge of the four species of *Plasmodium* although 80% of had not participated in a formal, organized malaria case management training session. Only 60% adhered to negative RDT results regardless of persistent symptoms of malaria. There is a need for initial and continuous training of HCW on malaria diagnosis using RDTs, differential diagnosis and unambiguous case management guidelines to increase their confidence in handling negative results and adherence to RDT results.

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ANALYSIS OF THE AGGREGATE AND DISTRIBUTIONAL WELFARE EFFECTS FROM VACCINE DIFFERENTIAL PRICING, POOLED PROCUREMENT AND POOL MEMBERSHIP

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A well designed vaccine pricing architecture can ensure more equitable vaccine prices and result in greater access to new vaccines globally. Pooled procurement is a mechanism that is commonly used to achieve lower vaccine (and medicine) prices for lower income countries and consequently allow country programs to immunize more people with a given budget. Under pooled procurement, several countries bargain collectively as one unit to achieve one (supposedly) lower price. Currently, there is very little (if any) analytical or empirical research to guide policy around the optimal buy-side market structure for vaccines. Existing vaccine procurement pools are organized in several different ways, each with different implications in terms lowering prices for countries in the pool, and for ensuring more equitable vaccine access globally. Some procurement pools, such as PAHO and GCC, are organized regionally (geographical proximity-based), with high income heterogeneity among the countries in the pool. Other pools such as UNICEF are organized by country income level (income-proximity based), but have to rely on a third party i.e. a UN or multilateral agency led procurement structure. Using game theoretical models this research attempts to answer the following questions: (1) What form of buy-side market structure (single purchasing pool vs. multiple purchasing pools; pools organized by income vs. pools organized by geo-spatial proximity; differential pricing within pools vs. single price within pools) maximizes overall social welfare and vaccine availability? ;(2) For each type of purchasing structure what is the distribution of welfare benefits across countries in different income groups?; and (3) What opportunities exist for improvement in the current organization of global vaccine pricing and procurement that will increase total social welfare, create more equitable distribution of welfare/benefits between the manufacturer(s), low income countries and middle income countries?

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A PROTOCOL TO OPTIMISE MICROSATELLITE DNA AMPLIFICATION OF *TRYPANOSOMA BRUCEI GAMBIENSE* FROM BODY FLUIDS

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Microsatellite genotyping of *Trypanosoma brucei gambiense*, the causative agent of human African trypanosomiasis or sleeping sickness, and population genetics tools, are useful for inferring population parameters such as population size and dispersal. Amplifying parasite DNA directly from body fluids (i.e. blood, lymph or cerebrospinal fluid) allows avoiding costly and tedious isolation phases. It is however associated to increased frequencies of amplification failures (allelic dropouts and/or null alleles). In this paper, we present a study focused on improving microsatellite loci amplification of *T. brucei gambiense* from Guinean sleeping sickness foci. We checked for the real nature of blank and of apparent homozygous genotypes of parasite DNA directly amplified from body fluids. We tested the effect of three different DNA quantities for different microsatellite loci of trypanosomes from different body fluids. Our results show that some initially blanks and homozygous genotypes happen to be actual heterozygous genotypes. In Guinea, lymph from the cervical nymph nodes, known to contain the highest concentrations of parasites, appeared to provide the best amplification results. Simply repeating the PCR may be enough to retrieve the correct genotype, but we also show that increasing initial DNA content provides better results while undertaking first amplification. We finally propose an optimal protocol for amplifying *T. brucei* DNA directly from body fluids that should be adapted to local characteristics and/or constraints.

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ECOLOGICAL NICHE MODELS FOR CUTANEOUS AND VISCERAL LEISHMANIASIS IN BRAZIL BASED ON MAXIMUM ENTROPY (MAXENT)

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Leishmaniasis are diseases of great medical, social, and economic importance in endemic areas and are considered serious public health problems due to its clinical impact and epidemiological diversity. They belong to the group of neglected diseases intrinsically associated with poverty as well as health iniquities. The goal of this work was to identify environmental and socioeconomic factors that may be associated with the occurrence of cutaneous (CL) and visceral (VL) leishmaniasis in Brazil from 2005 to 2009, using ecological niche models to predict the risk of disease at the municipality level. A GIS database was constructed using records of CL cases by municipality available in the national notifiable diseases information system (SINAN) database. Records from the Brazilian Institute of Geography and Statistics (IBGE) and the Pan-American Health Organization (PAHO) unsatisfied basic needs data for people (UBNp) and housing (UBNh)) were used as socioeconomic data variables. Environmental data included long-term normal monthly climate from WorldClim and MODIS remote sensing annual composite image data. Probability distribution models for CL and VL based on environmental and socioeconomic features were executed using Maxent and ArcGIS 10. From 2005 to 2009, a total of 96,351 cases of CL and 13,563 cases of VL were registered by SINAN. No cases of either disease were reported in 83% of municipalities; CL was reported in approximately 13% of the municipalities, mainly in the North and Northeast regions, and VL was reported in less than 1% of the municipalities and mostly in the Northeast. Maxent results showed that variables that contributed most to the environmental model for CL were precipitation of September (26.2%) and annual precipitation (17.3%)(AUC 0.80); for VL, precipitation in October (11.6%) and mean temperature of warmest quarter (14.5%) were the most influencing variables (AUC 0.948). The Maxent socioeconomic model was most influenced by the variables UNBp education (39.6%), UNBH plumbing (11.3%) and number of health units (8.8%) for CL (AUC 0.864) and the variables that most contributed in the VL scenario were human development index (25.7%), literacy rate (24%) and sewage services (18.9%) (AUC 0.928). Results suggest Maxent can be used to generate the probability distribution maps based on limited distribution point data and that models then can be used to improve the allocation of resources in control programs.

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ASSOCIATION BETWEEN DISSEMINATED LEISHMANIASIS AND POLYMORPHISMS IN *LEISHMANIA BRAZILIENSIS* STRAINS

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We have previously described a multiclonal population structure among genotypically polymorphic *Leishmania braziliensis* from an area with high endemicity for American tegumentary leishmaniasis (ATL) in Bahia, Brazil, named Corte de Pedra. Based on RAPD (*Randomly amplified polymorphic DNA*) profiles, we also found an association between clinical outcome of ATL and parasite genotypes in this region, indicating a role for the intra-species variability among these microorganisms on form of disease. In order to further explore the hypothesis of association between form of ATL and strain of *L. braziliensis*, we cloned, sequenced and compared homologous RAPD bands previously explored for genotyping the *L. braziliensis* of Corte de Pedra. With this strategy we found six genomic loci that were polymorphic between representatives of the different clades (i.e. subpopulations) of parasites described in that region. PCR primer sets were designed for the specific targeting of each locus identified. Using these primers each locus was re-amplified, electrophoresed and had the band corresponding to the amplicon gel extracted and cloned into pCRII vectors. Then six clones of each locus were sequenced per leishmania isolate. The cloned amplicons permitted identify that the SNPs and indels defining the polymorphisms at each locus segregate within the population of *L. braziliensis* in Corte de Pedra according to haplotypes. Several SNPs, indels and haplotypes displayed significant associations with disseminated leishmaniasis (DL). In particular, patients infected with *L. braziliensis* containing certain SNP genotypes and haplotypes found in the locus starting at position 425451 in chromosome 28 presented significantly increased risk ratios for developing DL. Thus this rapidly emerging form of ATL may have its outcome driven in part by the infecting *L. braziliensis* strain.

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ECOLOGY AND TRANSMISSION DYNAMICS OF VISCERAL LEISHMANIASIS IN ETHIOPIA: RESULTS OF A PROSPECTIVE STUDY TO DETERMINE HUMAN INFECTION RATES IN AN ENDEMIC AREA OF NORTH ETHIOPIA

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Globally, visceral leishmaniasis (VL), a systemic protozoan infection caused by *Leishmania donovani* spp. is estimated to afflict some 500,000 persons annually. In Africa, the worst affected regions are southern Sudan (15,000-20,000 cases/yr) and Ethiopia (4,000-5,000 cases/yr). VL is considered an emerging disease in north Ethiopia where it is associated with seasonal migration of agricultural laborers to endemic areas and HIV/AIDS. A prospective cohort study of VL in North Ethiopia was designed to elucidate the VL infection dynamics in an endemic setting. A cross-sectional survey was conducted around the town of Sheraro during March 2011 involving 4,883 individuals living in 18 villages. Participating households (1,386) were numbered and their coordinates were recorded. Demographic and socio-economic data were collected. Screening for VL by physical and laboratory examination was performed and previously treated VL cases (PTC) were noted. Exposure to *Leishmania* was assessed by Leishmanin Skin Test (LST). Sera and dried blood spots were tested by Direct Agglutination Test (DAT) and RT-PCR. The LST rate among 4,554 individuals was 10.1% and remained surprisingly low (35%) among 126 PTCs. Serological screening of 4,788 individuals by DAT identified 3.9% positives. Of 4,757 dried-blood samples tested by RT-PCR, 680 samples (14.3%) were found positive for *Leishmania* k-DNA. Of those, 119 (2.5%) harbored over 100 parasites per ml of blood. To validate these findings ITS1/PCR products were sequenced and 90% (19 of 21) were confirmed to be *L. donovani.* From March 2011 to February 2012, a total of 34 new VL cases (22 males, 12 females) were found amongst the study population. The mean age of these patients was 16. 8 (±12.5). Of these 34 cases, 38.2% were DAT positive in March 2011. Similarly, 15.6%, were positive by LST, and 27% were positive by k-DNA RT-PCR. Based on these data, the annual incidence of VL in the study localities is at least 7.0/1000. The study is ongoing, more data will accrue and the results of in-depth analysis will be reported.

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SAND FLY DENSITY REDUCTION IS LESS MARKED IN PRECARIOUS HOUSING AFTER INSECTICIDE THERMAL FOGGING

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Insecticide thermal fogging (ITF) is a tool to control vector borne diseases. It is generally assumed that ITF reduces vector density independently of housing conditions. Few studies have been focused on Sand Flies while also looking at housing characteristics. We conducted a 15 month longitudinal study that included two deltamethrin based ITF interventions in 12 of the 24 houses at Comunidad de Trinidad Las Minas, Capira, Panamá, an hyperendemic cutaneous leishmaniasis transmission village. During the study we followed sand fly (SF) abundance. We found a 50 to 80% reduction in SF density at fogged houses when compared with control houses, while controlling for seasonal changes in SF abundance associated with rainfall. We found some heterogeneities in the reductions, as abundance changed according to SF species, with *Lutzomyia gomezi, L. panamensis, L. dysponeta* and *L. triramula* reducing their density between 40% and 90% after ITF, in contrast to *L. trapidoi* whose density increased 5% after the ITF. Differences in the impact of ITF were associated with housing quality, the most precarious houses, i.e., those with features that ease insect entrance, had a disproportionally larger SF abundance, in some cases with an increased domiciliary SF density following the ITF. Our results suggest that ITF success potential to control SF density and Leishmaniasis transmission could be dependent on housing quality.

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THE ECO-EPIDEMIOLOGY OF *TRYPANOSOMA CRUZI* INFECTION IN RURAL COMMUNITIES OF THE HUMID CHACO OF ARGENTINA

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The eco-epidemiology of the domestic transmission of *Trypanosoma cruzi* in the humid Chaco region has seldom been investigated. We assessed the household distribution of bug, dog and cat infection in two local ethnic groups (Tobas and Creoles) and investigated differences in transmission risks between them, tested the role of domestic dogs and cats as reservoir hosts, and identified transmission risk factors. We conducted a cross-sectional survey of house infestation with *Triatoma infestans* bugs and *T. cruzi* infection in bugs, dogs and cats in a well-defined rural area in northeastern Argentina including 323 households.

Bug infection prevalence among 1,869 bugs examined was highest in domiciles (43%) and in storerooms and kitchens (24%), and was marginal elsewhere (<3%). The composite prevalence of infection was similar for 481 dogs (26%) and for 87 cats (29%) that were examined using serology and/or xenodiagnosis. Vector and host infections were highly aggregated at the household level. Using a catalytic irreversible model, the annual force of infection in dogs was three times higher in Toba than in Creole households, in agreement with other transmission indices. The demography of dogs and cats differed between ethnic groups in several respects. Random-coefficient multiple logistic regression analysis showed that infection in dogs increased significantly with age of the dog, number of infected dogs or cats in a household, and the relative abundance of infected bugs. The fraction of infected bugs increased significantly and in a dose-response fashion with number of infected dogs in a household. Infected cats also increased transmission to bugs when no infected dog was present. Our results reveal the persistence of domestic transmission of *T. cruzi* in northern Argentina, especially among Tobas; the occurrence of a peridomestic transmission cycle, and the key role of dogs and cats as domestic reservoir hosts, risk factors and control targets in the humid Chaco.

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CHAGAS DISEASE: KNOWLEDGE, ATTITUDES, AND PRACTICES AMONG LATIN AMERICAN IMMIGRANTS LIVING IN LOS ANGELES

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This study was undertaken to examine the knowledge, attitudes, and practices associated with Chagas disease (CD) among Latin American immigrants living in Los Angeles. Background: It is estimated that more than 300,000 individuals are infected with CD in the United States (US), though most were infected via vector-borne transmission in Latin America where it is endemic. We assessed the knowledge, attitudes, and practices associated with CD among 2712 individuals in Los Angeles County, ages 18 to 60 years, who had previously resided in Mexico, Central America, or South America for at least 6 months. Sixty-two percent of participants recall seeing triatomine insects in Latin America, and 27% of participants reported being bitten by triatomine insects at least once per year while living in Latin America. Eighty-six percent of participants had never heard of CD. These results were significantly affected by the participant's country of birth. Of the 13% of participants who had heard of CD, 62% thought CD was a problem in their native country, 55% thought CD was a problem in the US, and 81% thought CD was not serious. Over 90% of participants who had heard of CD would want to be tested and treated for it. In conclusion, the majority of Latin American immigrants residing in Los Angeles recall seeing the insects that transmit CD in their native country, yet they have never heard of CD. Of the participants who had heard of CD, the majority believe it is a problem in their native country and the US but do not believe it is a serious problem overall. Nevertheless, they would want to be tested and treated for CD.

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MODELING THE DISTRIBUTION OF CUTANEOUS LEISHMANIASIS IN BRAZIL BASED ON ENVIRONMENTAL AND SOCIOECONOMIC RISK FACTORS

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Cutaneous leishmaniasis (CL) presents a variety of casual agents, reservoirs and vectors with different transmission patterns. Out of the 12 new world species known to cause disease in humans, seven can be found in Brazil which makes control of this endemic difficult. Thus, the necessity of a new methodology which would consider a better definition of transmission and risk areas. This study aimed to model the distribution of CL at the municipality level in Brazil based on environmental and socioeconomic factors. The GIS database was constructed using records of CL cases available in the national notifiable diseases information system (SINAN from 2005-2009); records from the Brazilian Institute of Geography and Statistics (IBGE) and the Pan-American Health Organization (unsatisfied basic needs for people (UBNp) and housing (UBNh)) were used to compile the socioeconomic data. The environmental database was constructed using long-term normal monthly climate data from WorldClim and MODIS annual composite data. Distribution models for CL were executed using Maxent and maps of spatial distribution and prediction models were created in ArcGIS 10. A total of 96,351 cases of CL were registered at SINAN (13% of the municipalities). Cases of CL increased as the number of health facilities and UNBp education increased ($p<0.0001$) but notification of disease decreased as UNBh improved (drinking water; plumbing; sanitation and electricity ($p<0.0001$)). CL was inversely correlated with Temperature Seasonality ($p<0.0001$) and directly correlated with annual precipitation ($p<0.0001$). The environmental variables that most contributed in the Maxent model were precipitation of September (26.2%) and annual precipitation (17.3%) (AUC 0.80). From the socioeconomic data the most influencing variables were gross domestic product per capita (23%) and literacy rate (22%) (AUC 0.795, IBGE model); sanitation (83.9%; AUC 0.76, UBNh model); subsistence (33.7%) and unemployment (26%)(AUC=0.77, UBNp model). A final model was executed combining environmental and socioeconomic data and it was found that the variables contributing in the model were UBNh sanitation (39.6%), UBNp subsistence (11.3%) and annual precipitation (8.8%) (AUC 0.86). Socioeconomic factors may be playing an important role in the occurrence of CL in Brazil and together with environmental features may provide a better understanding of the dynamics of this endemic in Brazil.

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CONGENITAL TRANSMISSION OF *TRYPANOSOMA CRUZI* IN ARGENTINA, HONDURAS, AND MEXICO: AN ONGOING STUDY

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Trypanosoma cruzi has been divided into different lineages: *T. cruzi* I (TcI) and non-TcI (including lineages II-VI). TcI is predominant in Mexico and Central America, while non-TcI is predominant in most of South America, including Argentina. Little is known about congenital transmission of TcI. The specific aim of this study is to determine the rate of congenital transmission of TcI compared to non-TcI. We are conducting a prospective study to enroll at delivery, 10,000 women in Argentina, 7,500 women in Honduras, and 10,000 women in Mexico. We are measuring transmitted maternal *T. cruzi* antibodies by performing two rapid tests in cord blood (Stat-Pak, Chembio, Medford, New York, and Trypanosoma Detect, InBios, Seattle, Washington), and, if at least one of the results is positive, we are identifying infants who are congenitally infected by performing parasitological examinations on cord blood and at 4-8 weeks, and serological follow-up at 10 months. We will also perform standard PCR, real-time quantitative PCR, and *T. cruzi* genotyping on maternal venous blood and on cord blood, and serological examinations on siblings. Study

enrollment has been staggered and began in Tucuman, Argentina in April 2011, and in Intibuca and Santa Barbara, Honduras in May 2011. Study enrollment began in July 2011 in Merida and Valladolid, Mexico. As of April 2012, recruitment numbers per country are as follows: Argentina - 4,280 births; Honduras - 3,634 births (1,739 in Intibuca and 1,895 in Santa Barbara); and Mexico - 4,002 births (2,161 in Merida and 1,841 in Valladolid). Argentina has reported 80 (1.9%) births of seropositive mothers (with at least one positive serological rapid test result in cord blood), Honduras has reported 144 (4.0%) births of seropositive mothers (96 (5.5%) in Intibuca and 48 (2.5%) in Santa Barbara), and Mexico has reported 28 (0.7%) births of seropositive mothers (21 (1.0%) in Merida and 7 (0.4%) in Valladolid).

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GENETIC DIVERSITY AND POPULATION STRUCTURE OF *TRYPANOSOMA BRUCEI* IN UGANDA: IMPLICATIONS FOR THE EPIDEMIOLOGY OF SLEEPING SICKNESS AND NAGANA

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Human African Trypanosomiasis has remained a major and long term public health problem in Uganda characterized by recurrent sporadic outbreaks in the traditional endemics areas and continued spread to new unaffected areas. Uganda harbors the two forms of Trypanosoma brucei subspecies; *Trypanosoma bbrucei rhodesiense* and *T. b. gambiense* causing two forms of sleeping sickness, the acute and the chronic forms respectively. The third *T. brucei* subspecies; *T. b. brucei* is the third leading cause of African Animal Trypanosomiasis or nagana and has a wider geographical distribution. *T. b. gambiense* remains localized in North West Uganda while *T. b. rhodesiense* is currently restricted to Central and Eastern regions, although it continues to spread towards the *T. b. gambiense* foci. All the three forms of parasites are closely related subspecies and remain a major challenge to human health and animal production in Uganda. This is the only country where all three taxa occur. Thus, understanding the population structure of *T. brucei* in Uganda is critical for disease control. We use a newly developed set of microsatellite loci to investigate two important hypotheses regarding the population processes affecting *T. brucei* in Uganda: 1) Do temporally distinct disease foci originate from similar or distinct populations of *T. brucei*? 2) Does host species influence the genetic population structure of *T. brucei*? By investigating these hypotheses we aim to inform on evolutionary processes at the population level, which will assist in developing effective control measures and treatment of *T. brucei*. Results are based on isolate collections from 18 Ugandan sites including 300 *Trypanosoma* isolates from infected tsetse, vertebrates and humans.

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INFECTIOUSNESS OF SMALL RODENTS TO THE SANDFLY *LUTZOMYIA LONGIPALPIS* REINFORCES THEIR ROLE AS SOURCES OF INFECTION *LEISHMANIA (VIANNIA) BRAZILIENSIS*

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There are many records of leishmanial infections detected in wild animals by molecular methods but a major question is: Are they infectious to vectors? The present study was aimed at characterizing the infectiousness of experimental infections of *Leishmania* (*Viannia*) *braziliensis* in the small rodents *Necromys lasiurius, Rattus rattus* and *Nectomys squamipes*. These animals are incriminated as the major reservoirs hosts of cutaneous leishmaniasis in an endemic area of Pernambuco, northeast Brazil. For these experiments we established colonies of the three rodent species and a colony of *Lutzomyia longipalpis*. A total of 30 animals (10 *Rattus rattus*, 10 *Necromys lasiurius* and 10 *Nectomys squamipes*) and a control group of golden hamster *Mesocricetus auratus* were infected with cultured promastigotes of *L. (V.) braziliensis* (MBOL/BR/2000/CPqAM95): a stock previously isolated from the rodent *Bolomys lasiurus* (Syn. *Necromys lasiurus*) captured in the endemic study area, as reported previously. An average of 25 female sand flies was used to perform the xenodiagnosis. Ten days after feeding the sand flies were dissected and their intestinal tract was examined for the presence of promastigotes. Samples of the intestine were also preserved and were subsequently tested by Polymerase chain reaction (PCR) tests that were specific for the *Lutzomyia* cacophony gene and for the subgenus *L. (Viannia)* spp. Samples of skin, spleen and liver of each experimentally infected animals were tested by PCR for the presence *L. (Viannia)* DNA. Three *Necromys lasiurius*, 3 *Nectomys squamipes* and 5 *Rattus rattus* were infective for phlebotomine sand flies. The visualization of promastigotes in phlebotomine sand flies was confirmed by the PCR specific for the subgenus *L.(Viannia).* The results show that these 3 rodent species are infectious and strengthen their incriminated importance as natural reservoirs of *L. (V.) braziliensis*. They also indicate the potential use of molecular techniques to determine reservoir host infectiousness by comparing parasite load with xenodiagnoses results.

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THE SYLVATIC TRANSMISSION CYCLE OF *TRYPANOSOMA CRUZI* IN THE HUMID CHACO OF ARGENTINA

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A wide variety of wild mammals (e.g., marsupials, edentates, rodents and primates) are reservoir hosts of *Trypanosoma cruzi.* Understanding the complex epidemiology of *T. cruzi* and the variety of transmission cycles requires a representative picture of parasite genetic diversity – currently classified into six Discrete Typing Units (DTUs). We estimated the prevalence and diversity of *T. cruzi* infection in wild mammals of a well-defined rural area (Pampa del Indio) in Chaco, northern Argentina. A total of 195 mammals from 20 identified species were captured in four surveys conducted between 2008 and 2011 and examined for infection by xenodiagnosis and kDNA-PCR. A total of the 27 (14%) were xenodiagnosis-positive: 12 of 31 (29%) *Didelphis albiventris* opossums and among armadillos, 12 of 29 (46%) *Dasypus novemcinctus*, 2 of 15 (13%) *Tolypeutes matacus*, and one of 16 (6%) *Chaetophractus*

vellerosus. A total of 15 xenodiagnosis-negative animals were kDNA-PCR-positive, including 4 D. albiventris opossums, 1 *Euphractus sexcinctus* and 3 *D. novemcinctus* armadillos, 5 *Thylamys pusilla* (Chaco fat-tailed opossum), and 2 small rodents (unidentified species). Using SAT-DNA-PCR we confirmed *T. cruzi* infection in one *D. novemcinctus* and two *T. pusilla* positive by kDNA-PCR only. These are the first findings of *T. cruzi* in *T. pusilla* and TcIII in *C. vellerosus* and *T. matacus* from Argentina. A PCR-based strategy showed that all opossums were infected with DTU TcI and all armadillos with TcIII, implying separate parasite transmission cycles. Wild mammals had no evidence of parasite DTUs infecting local domestic dogs, cats or *Triatoma infestans* bugs (TcV and TcVI). Sylvatic transmission cycles of *T. cruzi* in the dry and humid Chaco differ in the composition of the main reservoir hosts.

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LEISHMANIA BRAZILIENSIS IS THE ETIOLOGICAL AGENT OF CUTANEOUS LEISHMANIASIS IN LOS MONTES DE MARÍA, COLOMBIA

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Cutaneous leishmaniasis (CL) and visceral leishmaniasis (VL) constitute important public health problems in the Caribbean coastal region of Colombia. These clinical presentations of leishmaniasis are endemic in rural and urban areas of the departments of Sucre and Bolívar, especially in Los Montes de María, an area that constitutes the most important CL macrofocus of the Caribbean coastal region. The objective of the present study was to use sequencing of the subtelomeric region to determine the *Leishmania* species producing CL among the inhabitants of Montes de María. Thirty-six CL patients from the municipalities of Carmen de Bolívar, Macayepo, Morroa, Sincelejo and Ovejas were analyzed, each receiving a direct parasitological examination before samples were taken for parasite culture in NNN medium to allow molecular identification of the species involved. A sequence from the subtelomeric region of approximately 388 bp was obtained, presenting a 99-100% similarity with sequences of the subtelomeric region of three reference strains of *Le. (V.) braziliensis.* It was thus determined that is the species responsible for CL in the Montes de María area. Its presence in the area has important implications in selecting the correct medical treatment to be administered.

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HEALTHY LIVING TO CONTROL CHAGAS DISEASE IN ECUADOR

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Chagas disease is caused by the parasite *Trypanosoma cruzi* and transmitted mainly by the feces of triatomine insects. This disease affects ~10 million people mostly in Latin America. Studies conducted since 2002 by our group have described the biological and epidemiological factors that facilitate transmission of *T. cruzi* in Southern Ecuador, and have determined that insecticide-based control strategies are effective only in the short term due to frequent bug re-infestation of treated dwellings. To address this issue, we designed in 2010 the Healthy Living Initiative (HLI), a holistic process aimed at facilitating the socioeconomic development of rural communities affected by Chagas disease. The final goal of the HLI is to design, implement and evaluate a sustainable model to eliminate vectorial Chagas disease transmission in Loja province via improvement of the houses and the peridomestic areas. This model is based on community organization and socioeconomic participative development as basic conditions to promote human health. So far it has been possible to facilitate process in five areas: health (community promoters and entomological surveillance network); infrastructure (land entitlement and improvements to local water systems and access road); income generation (ecotourism, artisans' groups, and local products commercialization); capacity building, and safety/security. Based on these advances, the current phase of the HLI identifies characteristics of a Healthy Housing Model adapted to the cultural and social realities of this area. Positive Deviance methodological framework was used with particular attention to existing knowledge, attitudes, and practices (KAP) of houses that have remained bug free during the last four years. In this process the HLI seeks to unite the efforts of various local, national and international organizations active in Loja by integrating their activities to government plans, as well as facilitating families' participation through critical analysis of their own reality.

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EPIDEMIOLOGY OF ACTIVE *TRYPANOSOMA CRUZI* TRANSMISSION AND IMPACT OF INSECTICIDE SPRAYING IN AREA IN THE BOLIVIAN CHACO

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An estimated 8-10 million people are infected with Chagas disease. Prevention strategies rely primarily on insecticide spraying against vectors and risk factor reduction including housing improvements. We performed a cross-sectional survey to evaluate the prevalence and risk factors for disease acquisition in seven contiguous villages in an area of the Bolivian Chaco where active transmission persists despite an insecticide spraying program that operated from 2000-2007. Furthermore, we attempted to evaluate the effectiveness of the insecticide spraying program by modeling disease incidence on age-specific prevalence using a catalytic model. Survey teams performed a census of the 7 evaluated villages, and collected demographic, socio-economic, and risk factor data. We collected venous blood from 1578 persons aged ≥ 2 years, and performed Indirect Hemagglutination and Weiner ELISA on each sample for *Trypanosoma cruzi* diagnosis. Discordant results were confirmed by Weiner recombinant antigen ELISA. The population prevalence of *T. cruzi* infection was 51.8%. We limited our analyses of risk factors to the ≤15 year age group, in which prevalence was 19.5%, and assumed that infection was acquired relatively recently. Preliminary univariate analyses demonstrated statistically significant associations between *T. cruzi* seropositivity and village of residence ($P < 0.0001$), roofing material of metal as compared to straw (Odds ratio [OR] 0.57; 95% Confidence Interval [CI]: 0.33-0.99), and household ownership of ducks (OR 1.77; CI: 1.22-2.57). No association between serostatus and history of household insecticide spraying (OR 1.03; CI: 0.63-1.67) was found in univariate analysis, and no significant decrease in risk of infection associated with the spraying campaign was detected in the catalytic model ($\chi^2(1) = 0.42$; $P = 0.52$). Failure of spraying to yield a decrease in transmission may be due to inadequate spraying, insufficient duration or frequency of the program, insecticide resistance, or reinfestation by sylvatic vectors. Multivariate analyses are forthcoming.

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SPATIOTEMPORAL CLUSTERING OF VISCERAL LEISHMANIASIS AND *LEISHMANIA DONOVANI* INFECTIONS IN BIHAR, INDIA

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In the Indian subcontinent, visceral leishmaniasi (VL), also known as kala-azar, is caused by *Leishmania donovani*, which is transmitted from man to man by the sand fly Phlebotomus *argentipes*. VL tends to cluster in certain hamlets in remote rural villages but the spatiotemporal dynamics of the disease and leishmania infection are not fully understood. We analysed the clustering of VL cases and *L. donovani* infections in a VL endemic area covering over 80,000 people in Muzaffarpur district, Bihar. The people living in the study area are regularly monitored and demographic information is been gathered as part of NIH funded project on VL in India. VL cases occurring from 2007 to 2011 were identified by yearly house to house surveys in the whole study area. Incident *L. donovani* infections were identified as seroconverters (using DAT and rK39 ELISA) in high transmission areas by means of two serosurveys in 2008 and 2009 (n=11,000 people). Yearly edge-corrected kernel density maps, the K-function and the scan-statistic were used to evaluate the spatiotemporal dynamics of VL and *L. donovani* infection over the study period. The implications of VL clustering and spatial variation for the VL control program in the Indian subcontinent will be discussed.

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EXAMINING LEVEL OF USE OF CHEMOTHERAPY, CHEMOPROPHYLAXIS AND INTERMITTENT-PREVENTIVE TREATMENT OF MALARIA IN PREGNANCY BY PREGNANT WOMEN IN NIGERIA

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The study assessed the nature of health seeking for chemotherapy and chemoprevention for malaria-in-pregnancy (MIP), especially the acceptability and use of intermittent preventive treatment of MIP by pregnant women attending public and private health facilities. The study was undertaken in Enugu, southeast Nigeria. A total of 647 consenting pregnant women (321 in the public hospitals and 326 in the private hospitals) were administered with structured questionnaires. Data was analyzed for the levels of perceptions, acceptability and use of IPTp amongst the pregnant women. Bivariate analysis was used to examine whether the differences in the variables between pregnant women attending public and private facilities were statistically significant. The results showed that the knowledge about MIP was high among the pregnant women. Pregnant women attending private hospitals were less aware of IPTp as a preventive strategy for MIP ($p<0.05$), but there was no significant difference in the acceptability of IPTp by the pregnant women in public and private facilities ($p>0.05$). IPTp was consumed more by pregnant women in the private facilities (76.9%) compared to those in the public facilities (27.6%) ($p<0.05$). Blood tests were used more by consumers in the private facilities (71.3%) compared to those in the public facilities for diagnosis of MIP (50.2%) ($p<0.05$). It is concluded that health seeking behaviour for MIP by pregnant women attending private facilities was better than for those attending public facilities. Hence, interventions are needed to improve the management of MIP in public facilities, and also enhancing the services of private providers.

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DESCRIPTIVE SURVEILLANCE ON USE OF ARTEMETHER-LUMEFANTRINE IN PEDIATRIC AND ADULT RETURNING TRAVELERS WITH MALARIA

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Data from clinical studies show that artemether-lumefantrine (AL) is effective and well tolerated in children and adults with uncomplicated *Plasmodium falciparum* malaria. However, data on effectiveness and safety of AL in patients in non-endemic settings are limited. Our 5-year prospective surveillance plan includes AL-treated pediatric and adult patients with suspected or confirmed *P. falciparum* malaria in the US, as reported to the National Malaria Surveillance System at the Centers for Disease Control and Prevention. Descriptive analyses include demographics and baseline characteristics, including malaria immune status; treatment effectiveness; prior and concomitant medication use; and occurrence of adverse events. In the first 17 months (1 May 2010 to 30 September 2011), demographics, treatment adherence, and safety data were collected on 24 patients. Treatment effectiveness data were collected on 21 (91.3%; 2 patients were lost to follow-up) of 23 patients with confirmed (smear 95.7%, PCR 4.3%) or suspected malaria. The mean age of patients was 40.3 years (SD=19.3; range 12-83) and the median BMI was 27 kg/m^2 (range 16.8-33.8). The majority were male (58.3%) and malaria non-immune (91.7%). Half were non-Hispanic Black. The most common malaria species was *P. falciparum* (65%; others were *P. vivax, P. ovale,* and *P. malariae*, 22%; undertermined, 13%). Of 22 patients taking AL, 18 (81.8%) adhered to treatment. The overall cure rate of patients treated with AL was 83.3% (95% CI= 58.6-96.4%) on Day 7 and 82.4% (95% CI=56.6-96.2%) on Day 28 (patients with missing effectiveness data excluded from analysis). The most common prior and concomitant medications included analgesics, other antimalarials, vitamins, and supplements. There were no deaths, but 3 serious adverse events (severe malaria, incorrectly diagnosed as uncomplicated malaria) were reported. Treatment of *P. falciparum* malaria in non-immune patients with AL is effective and well tolerated without any unexpected or new safety findings with approved 3-day treatment regimen.

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EFFICACY OF SHORT PROPHYLACTIC COURSE OF ATOVAQUONE- PROGUANIL

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Current guidelines recommend continuation of Atovaquone proguanil hydrochloride (AP) prophylaxis for seven days after leaving *Plasmodium falciparum* endemic areas. Evidence from previous studies suggest that continuation for one day after malaria exposure ends may be sufficient. We conducted a retrospective survey of travelers who terminated AP prophylaxis one day after leaving malaria endemic areas to identify *falciparum* malaria cases. A retrospective telephone survey of travelers to sub-Saharan Africa. Travelers who visited our pre-travel clinic and used AP prophylaxis were included. After returning from their trip, travelers were contacted and questioned regarding prophylaxis adherence, duration, and malaria diagnosis during or after travel. In Israel, malaria is a reportable disease. A retrospective analysis was performed looking at all *falciparum* malaria cases reported to the Israeli ministry of health (MOH) between 2003-2008. Information about prophylaxis use among these patients were retrieved. The survey included 454 travelers between the years 2010-2011 (total 4771 days in endemic areas). AP was discontinued one day after leaving the malaria endemic areas by 341/454 (75%) travelers. No cases of malaria were noted. The MOH registry survey included 118 *falciparum* patients between the years 2003-2008. The majority (100; 85%) did not

take any malaria prophylactic. None of the patients had used malaria prophylaxis with AP (neither regular nor short AP course). Between 2005-2007, 2095 travelers to Sub-Sahran Africa consulted the Sheba Medical Center pre-travel clinic (total travel days to Sub-Sahran Africa = 134,488). There were no reports of malaria among these travelers. In conclusion, we retrospectively studied a large group of travelers exposed to highly endemic malaria areas. Despite cessation of AP prophylaxis one day after leaving the endemic areas none of the travelers developed malaria. In addition, analyzing the passive surveillance data of malaria cases in Israel did not show any *falciparum* malaria case which occurred after AP prophylaxis (regular or short course). Based on pharmacokinetic properties and *falciparum* malaria pathophysiology it is reasonable to recommend use of AP prophylaxis ending one day after leaving the endemic area. Further prospective validation of our findings in larger number of travelers should follow.

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IMPACT OF INTERMITTENT PREVENTIVE TREATMENT IN PREGNANCY WITH SULFADOXINE-PYRIMETHAMINE ON PLACENTAL INFECTION AND INFANT BIRTH OUTCOMES IN MALAWI

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Malaria in pregnancy is associated with severe maternal anemia, placental parasitemia, low birth weight, and increased perinatal mortality, especially among primi- and secundi-gravidae. Sulfadoxine-pyrimethamine (SP) is currently recommended for intermittent preventive treatment in pregnancy (IPTp), despite increasing prevalence of SP resistance that might compromise its effectiveness. HIV-uninfected women with a singleton pregnancy were enrolled at delivery and data on number of SP doses during the pregnancy collected via interview and review of the woman's antenatal care card. The primary outcome was evidence of past or current placental infection by placental histology. Secondary outcomes included malaria parasitemia at the time of delivery in the cord blood, placenta or maternal peripheral blood, and composite birth outcome (any of small for gestational age (SGA) as assessed by Ballard exam, low birth weight, or preterm). Of 713 women enrolled, 22% received <2 SP doses; 33% were primigravid. About one-third reported sleeping under a bednet the previous night. Receipt of <2 SP doses versus ≥2 doses had no impact on placental infection as measured by placental histology (31.5% vs 31.8%, P=0.94) or blood films (3.8% vs 5.9%, P= 0.30) at the time of delivery. Receipt of IPTp-SP was associated with a dose dependent protective effect in primigravid women only on the composite birth outcome due to a reduction in SGA; using 0 doses as the comparison, adjusted prevalence ratio (aPR) =0.69 (95% confidence interval (CI) 0.5-1.01), aPR=0.43 (95% CI 0.3-0.6), and aPR=0.32 (95% CI 0.1-0.9) for 1, 2, and 3 doses, respectively. Receipt of SP was not associated with stillbirths or adverse delivery outcomes. IPTp-SP did not reduce placental infection, but was associated with improved birth outcomes in primigravidae in Malawi, suggesting that IPTp-SP may work primarily by treating infection, rather than prophylaxis. Very few women received 0 doses of SP, so these results may underestimate the true effect of IPTp-SP. IPTp-SP should continue to be provided to pregnant Malawian women, but given the high prevalence of SP resistance in Malawi, alternative antimalarials should be investigated for IPTp.

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EFFICACY, SAFETY AND TOLERABILITY OF DIHYDROARTEMISININ-PIPERAQUINE FOR TREATMENT OF UNCOMPLICATED *FALCIPARUM* MALARIA IN PREGNANCY IN GHANA: A RANDOMIZED, NON-INFERIORITY TRIAL

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Pregnancy-associated malaria remains a challenge in endemic areas, resulting in low birth weights, maternal anaemia, foetal loss and increased infant morbidity and mortality. A major intervention recommended is effective case management with artemisinin-based combination therapy. One such option is dihydroartemisinin-piperaquine for which there is limited efficacy and safety data in pregnancy. With its introduction on the Ghanaian market and anticipated access by pregnant women, we assessed its use for treatment in pregnant women. Pregnant women of gestation15-32 weeks attending antenatal clinics in a moderate-to-high transmission zone of Ghana were screened for peripheral *falciparum* parasitaemia using rapid diagnostic test (RDT) and microscopy. Those positive in both and eligible were randomized to receive dihydroartemisinin-piperaquine or artesunate-amodiaquine. Baseline clinical, haematological and ultrasonographic assessments are conducted. They are actively followed up on days 1, 2, 3, 7, 14, 28, 42, at delivery and at 6 weeks postpartum to ensure adherence to study drugs, assess adverse events, collect blood samples for haematological and parasitological assessments and gather data on neonatal morbidity and mortality. Of a sample size of 904, 254 (28.1%) have been recruited giving a baseline RDT positivity of 14% (254/ 1805) and mean haemoglobin concentration of 10.0mg/dl. Of the day 3 blood films, 23.4% (43/ 184) had parasitaemia while 7.9% (14/177), 1.0% (2/193), 2.3% (4/172) and 0.6% (1/154) of the day 7,14, 28 and 42 blood films respectively showed parasitaemia. Geometric mean parasite density decreased from 147/ul(CI 134, 162) at baseline to 88/ul(CI 71, 109) on day 3. Polymerase chain reaction work on filter paper blots to differentiate reinfection and recrudescence is yet to be done. Upon completion of the study, we will compare parasitological efficacy at days 28 and 42, low birthweight, maternal haemoglobin, adverse events and foetal loss in the two treatment arms for significance of differences.

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MALARIA CASE MANAGEMENT PRACTICES IN PHARMACIES AND LICENCED CHEMICAL SHOPS IN GHANA

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Malaria is the single most important cause of morbidity and mortality in Ghana, especially among children under five years. Among pregnant women, malaria accounts for 28.1% of OPD attendance in Ghana. About half of the people first seek help from the pharmacies and licensed chemical sellers (LCSs) when they are sick. This study sought to assess the practices of pharmacies and LCSs with respect to malaria in Ghana. A random sample of 216 pharmacies and 306 LCSs were drawn from the register of pharmacies and LCSs in Ghana. A mystery client approach was employed in gathering information. The interviewers who acted as mystery clients were trained using a structured mystery client guide. Scenarios of children under five years, adults and pregnant women with malaria were presented at the pharmacies and licenced chemical shops, based on which purchases of ACTs were made. The data was captured in epidata and analyzed in SPSS. About 63% of LCSs and 56.9% of pharmacies asked the clients about the age of the patient (child under-five, pregnant women and other adults). About 54% of LCSs and 57% of pharmacies visited asked about the symptoms of the patient. Also, 26% of LCSs and 35% of pharmacies asked about the medication history of the patient. About 88% of LCSs and 90% of pharmacies visited recommended some drugs to the

clients after they had presented their symptoms. The rest did not. The rate of purchase of anti-malarials was not associated with the type of facility. The same proportion of LCSs and pharmacies (i.e. 2.3%) referred the clients to a clinic for diagnostic tests. Management practices of pharmacies and LCSs were encouraging however most of them did not ask about the medication history of the clients. Pharmacy council of Ghana should educate the pharmacies and LCSs on the need to ask their clients about the medication history of their illness before prescribing anti-malarials to them.

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HOW PATIENTS TAKE MALARIA TREATMENT: A SYSTEMATIC REVIEW OF THE LITERATURE ON ADHERENCE TO ANTIMALARIALS

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Artemisinin-based combination therapies (ACTs) are the first-line drugs for treatment of malaria throughout sub-Saharan Africa, and are becoming increasingly available in the private sector. However, there are concerns about sub-optimal patient adherence which may have consequences for treatment efficacy and the development of antimalarial drug resistance. In order to identify patterns in how patients use antimalarial drugs and highlight gaps in current knowledge, a systematic literature review was performed. A search was conducted in PubMed using MeSH and free text terms. Of 1242 studies initially identified, 40 met the inclusion criteria of providing quantitative data on patient adherence to antimalarials obtained for treatment. Manual search of reference lists and contacting researchers in the field yielded 11 additional studies. Patient adherence to ACTs was assessed in 23 studies, non-artemisinin-based combinations in 12, and chloroquine and other monotherapies in 20. Only two studies involved the private sector. Adherence measurement methods included self-report with and without dose timing, pill counts and biological assays. Although some studies found very high adherence to ACTs, others endeavouring to capture "real life" situations reported adherence of 64-88%. Overall, adherence was higher in studies where consent was obtained at enrolment versus at follow-up, and in studies where patient consultations were observed by the study team. Comparison of results based on different measurement methods showed higher adherence when biological assays were used, but no other clear patterns. Multivariate models in 10 studies found 28 factors associated with adherence, but no factor was significant in more than one study. The suboptimal patient adherence to ACTs obtained in the public sector and the current dearth of data from the private sector represent significant challenges to ensuring ACTs are used appropriately and remain effective. To strengthen future studies, there is a clear need for awareness of the impact of study procedures on adherence outcomes, and the identification of improved measurement methods that are less dependent on self-report.

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SONTOCHIN AS A GUIDE TO DEVELOPMENT OF DRUGS AGAINST CHLOROQUINE RESISTANT MALARIA

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Sontochin was the original chloroquine replacement drug, arising from research by Andersag two years after chloroquine (known as "Resochin" at the time) had been shelved due to the mistaken perception that it was too toxic for human use. We were surprised to find that sontochin, i.e., 3-methyl-chloroquine, retains significant activity against chloroquine-resistant strains of *Plasmodium falciparum in vitro*. We prepared derivatives of sontochin, "pharmachins", with alkyl or aryl substituents at the 3-position and with alterations to the 4-position side chain to enhance activity against drug resistant strains. Modified with an aryl substituent in the 3-position of the 7-chloro-quinoline ring PH-203 exhibits low nanomolar IC_{50} values against drug sensitive and multidrug resistant strains and *in vivo* efficacy against patent infections of P. yoelii in mice that is superior to chloroquine. Our findings suggest that novel 3-position aryl pharmachin derivatives have the potential for use in treating drug resistant malaria. A detailed structure-activity profile will be presented.

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WEIGHT BASED DOSING CAUSES SIGNIFICANTLY LOWER CHLOROQUINE CONCENTRATIONS IN YOUNGER CHILDREN

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Chloroquine (CQ) was previously the main drug for treatment of *Plasmodium falciparum* malaria but it is no longer recommended due to resistance. In Guinea-Bissau, West Africa double dose CQ (50mg/kg) split into 2 daily doses for 3 days was well tolerated, as efficacious as artemether-lumefantrine and eradicated 87% of *P. falciparum* with resistant genotypes in 2008. As part of 3 previous clinical trials, 100 µl of blood was collected on day 7 from children aged 6 months-15 years that had taken 25 or 50 mg/kg of CQ. Whole blood CQ concentrations were analysed using high performance liquid chromatography. CQ concentrations were obtained from 188 and 293 children after intake of 25 and 50 mg/kg of CQ, respectively. CQ concentrations after intake of 25 mg/kg and stratification by age were: 545 (10y) nmol/l. Using the same age groups, concentrations after 50mg/kg were 834, 1220, 1164, 1573, 1565 and1546 nmol/l. The increases with age were significant, nonparametric test for trend P=0.008 and P<0.0001, respectively. Using the same age groups, the dose of CQ taken according to body surface area ranged from 489-702 and 978-1405 mg per square meter after intake of 25 and 50 mg/kg, respectively. The CQ concentration in children 10 years of age after intake of 25 and 50 mg/kg, respectively. CQ concentrations were only 21% higher in children <2 years taking 50mg/kg compared to children >10 years taking 25mg/kg. Dosing according to body weight rather than body surface area most probably accounts for the lower concentrations seen in younger children. Vomiting and spitting among the youngest children are unlikely explanations as treatment was repeated after vomiting and because it does not explain the trend throughout the age groups. Chloroquine should be dosed according to body surface area and the effect of dosing according to body weight should be assessed for other antimalarials.

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RANDOMIZED, CONTROLLED TRIAL OF TREATMENT OF FEBRILE CHILDREN WITH A NEGATIVE MALARIA RAPID DIAGNOSTIC TEST WITH ARTEMETHER-LUMEFANTRINE VS. NO ANTIMALARIAL IN BAGAMOYO DISTRICT, TANZANIA

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Until recently, most national malaria control programs recommended treating all febrile children less than five years of age with first-line antimalarial drugs to prevent severe malaria, disability and death. In 2010, WHO recommended uniform confirmation of malaria parasitemia by blood smear or rapid diagnostic test (RDT) prior to treatment. Unfortunately

little is known about the epidemiology of other common causes of non-malarial fevers in malaria endemic areas. Moreover, the effect of withholding malaria treatment from febrile children, even when they test negative, may have unintended public health consequences. From January 2010 to December 2011, we enrolled 1000 children aged 6 to 59 months with uncomplicated febrile illness and negative malaria RDTs from two health facilities in Tanzania. Subjects were randomized to receive either artemether-lumefantrine (AL) or no treatment and followed for 91 days to document symptom resolution, time to next malaria infection, and frequency of hospitalization or death. Subjects who missed more than two follow-up visits were not included in per protocol analyses. Preliminary results are available for 708 (70.8%) subjects. Among these 708 subjects, 353 (49.9%) were randomized to AL, 457 (64.6%) completed 91 days of follow-up per protocol without developing malaria, 15 (2.1%) were healthy to day 91 but missed more than two visits, 14 (2.0%) withdrew consent, 8 (1.1%) were given a non-study antimalarial, 118 (16.7%) were lost to follow-up, 93 (13.1%) developed malaria during follow-up, and 3 (0.4%) died of non-study related illness. Children randomized to receive AL had a lower risk of developing malaria during follow-up (RR=0.72, 95% confidence interval 0.49-1.04, p=0.09). The time to malaria infection by 10% of subjects in each arm was 56 days for the AL arm and 28 days for those who did not receive treatment, but the difference was not statistically significant (p=0.07). Data are preliminary. Study results will be used to improve the management of non-malarial febrile illness.

837

AN INHIBITOR OF MULTIPLE CYTOCHROME P450S, 1-AMINOBENZOTRIAZOLE, ALTERS THE PHARMACOKINETICS OF PRIMAQUINE AND CHLOROQUINE IN A *RHESUS* MODEL OF MALARIA RADICAL CURE

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An unidentified metabolite of primaquine (PQ) is suspected to exert anti-hypnozoite activity to prevent *Plasmodium vivax* relapse. We previously showed that a nonselective inhibitor of multiple cytochrome P450s (CYP450s), 1-aminobenzotriazole (1-ABT), blocks PQ's malaria causal prophylaxis activity in mice. Subsequently, we attempted to use this inhibitor to explore if CYP450 metabolism is involved in PQ's anti-relapse activity in *P. cynomolgi*-infected Rhesus monkeys. Infected monkeys were administered 1-ABT prior to treatment with a 7 day radical curative regimen of PQ plus chloroquine (CQ). Efficacy +/- 1-ABT administration was determined via daily parasitemia readings and safety was assessed using clinical laboratory results, including % methemoglobin (metHgb). The 7 doses of 1-ABT and primaquine planned were halted after the second dose because some monkeys had elevated alanine aminotransferase levels, which returned to baseline after stopping dosing. Increases in metHgb occurred only in monkeys treated with PQ plus CQ. In contrast, metHgb decreased daily in animals pre-dosed with 1-ABT, suggesting 1-ABT blocks PQ-induced metHgb formation. Blood draws were included to assess plasma pharmacokinetics (PK) of PQ and CQ +/- 1-ABT. Pre-treatment with 1-ABT decreased PQ and CQ levels and prolonged half-lives. Animals pre-dosed with 1-ABT had a 4 to 7 day delay in onset of malaria relapse, relative to controls given CQ only, presumably because 1-ABT inhibits metabolism of the antimalarial active CQ parent drug. Besides PK interactions of 1-ABT with PQ and CQ, we noted a link between PQ and CQ metabolism. Animals given PQ plus CQ had a two-fold greater plasma exposure to CQ's major CYP450 metabolite (desethyl-CQ) after 1 dose and 8-fold higher levels after 7 daily doses relative to animals given CQ alone. In contrast, pre-dosing with 1-ABT precluded formation of desethyl-CQ. We report that the CYP450 inhibitor, 1-ABT, alters PK properties of PQ and CQ, and that PQ potentially induces CQ metabolism. Our results also suggest PQ metabolism is linked to metHgb generation.

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EXPLORING THE PERFORMANCE OF RETAIL SECTOR PROVIDERS EXPOSED TO A PILOT INTERVENTION TO PROVIDE SUBSIDIZED ARTEMETHER-LUMEFANTRINE THROUGH THE PRIVATE SECTOR IN WESTERN KENYA

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To improve the quality of care received for presumptive malaria from the highly accessed private retail sector in Western Kenya, subsidised pre-packaged artemether-lumefantrine (AL) was provided on a pilot basis to private retailers, together with a one day training for retail staff on malaria diagnosis and treatment. The pilot was assessed using a cluster randomised, controlled design with nine control and nine intervention sublocations, equally distributed across three districts. Provider and mystery shopper cross-sectional surveys were conducted at baseline and eight months post intervention to assess the impact of the intervention on retailer performance. Data were analysed based on cluster-level summaries, comparing control and intervention arms, while adjusting for covariates, including baseline values where a difference of 5% or more was observed between the arms at baseline. On average 564 retail outlets were interviewed per year. At follow up, 43% and 1% of respondents reported that at least one staff member had attended the intervention training, in the control and intervention arm, respectively. The intervention significantly increased the percentage of providers knowing the first line treatment for uncomplicated malaria by 24.2% points (confidence interval (CI):14.8%, 33.6%; adjusted p=0.0001); the percentage of outlets stocking AL by 31.7% points (CI: 22.0%, 41.3%; adjusted p=0.0001); and the percentage of providers prescribing AL for presumptive malaria by 23.6% (CI:18.7%, 28.6%; adjusted p=0.0001). No significant difference was observed between the arms at endline in the percentage asking for specified danger signs that determine need for referral to a health facility, and there remained substantial room for improvement in provision of appropriate dispensing advice to those who bought AL. Overall, subsidizing ACTs and retailer training can significantly increase the percentage of outlets stocking and selling AL for the presumptive treatment of malaria, but further research is needed on strategies to improve the provision of counselling advice to retail customers.

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COMPARATIVE EFFICACY AND ACCEPTABILITY OF ARTEMETHER-LUMEFANTRINE VERSUS DIHYDROARTEMISININ-PIPERAQUINE IN KENYAN CHILDREN WITH UNCOMPLICATED *FALCIPARUM* MALARIA

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The primary objective was to compare the corrected Acceptable Clinical and Parasitological Responses (ACPR) on Day 28 of artemether-lumefantrine (AL) and dihydroartemisinin-piperaquine (DP) in children with uncomplicated *falciparum* malaria. This open-label, comparative trial study in Western Kenya randomized 454 children with uncomplicated *falciparum* malaria of age 6-59 months to receive either AL (n=227) or DP (n=227). Children were hospitalized for 3 days for observed treatment and 72-hour parasite kinetic monitoring, and actively followed up at scheduled visits after discharge from hospital on Days 7, 14, 28 and 42. Genotyping for determining treatment outcome was performed on Day 0 and any other day the study participant had a recurrence of parasitemia. No significant differences were observed for the corrected ACPR rates on Day 3, 14, 28 and 42 for AL (99.1%, 100%, 97.8%, 96.8%) and DP (100%, 100%, 99.1%, 98.7%). Similarly, for the uncorrected ACPR rates no significant differences were seen on Day 3, 14, 28 and 42 for AL (99.1%; 98.7%; 81.1%; 67.8%) and DP (100%; 100%; 87.7%; 70.5%), ($p>0.05$ for all comparisons). Both AL and DP are efficacious treatments for uncomplicated *falciparum* malaria in Kenyan children. No signs of *P. falciparum* resistance to artemisinins were noted.

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DRUG-DRUG INTERACTIONS BETWEEN PRIMAQUINE AND CHLOROQUINE: PHARMACOKINETIC AND TRANSPORTER INHIBITION STUDIES

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The long established potentiation of primaquine's liver stage activity when co-administered with chloroquine is still poorly understood after more than six decades (Alving et al., 1955). In the present study we have compared the pharmacokinetics of primaquine (8.8 mg/kg PO in C3H mice) and its primary plasma metabolite carboxyprimaquine after co-administration of 90 mg/kg of chloroquine (CQ). The overall effect observed was a decrease in C_{max} with a corresponding decrease in Cl/F and increase in AUC. To better understand these effects, transporter inhibition studies were carried out using both MDR1-MDCK and Caco2 cell lines. Permeability experiments with increasing levels of CQ showed a marked dose dependence in B-A permeability, indicative of MDR1 inhibition. Results for a larger screen of the effects of CQ on various efflux and uptake transporters will be presented.

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INHIBITORS OF PRIMAQUINE METABOLISM AS MODULATORS OF EFFICACY AND HEMOLYTIC TOXICITY

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The 8-aminoquinoline drug primaquine (PQ) is the only drug approved for the treatment of relapsing relapsing malaria. However, PQ is known to cause hemolytic toxicity in G6PD deficient individuals. Proposed mechanisms of both efficacy and toxicity suggest a role for transient reactive oxygen species formed as a byproduct of metabolism. We previously showed that CYP 2D6 plays a major role in the production of the redox active metabolites most likely to produce oxidative stress, however the relevance of this role *in vivo* was not clear. To this end, the effects of Paroxetine (PX), a potent selective inhibitor of CYP 2D6 metabolism, co-administration was tested in models of both *in vivo* liver stage efficacy and G6PD deficient hemolytic toxicity. In C57BL/6 mice, co-administration of PX at 25 mg/kg with PQ at 2.5 mg/kg showed a reduction in liver stage potency at both 24 and 48 hr post infection with P. berghei sporozoites. Co-administration of PQ with the MAO-A inhibitor Clorgiline (CG) however, resulted in enhanced liver stage efficacy. Further, significant mitigation of the hemolytic toxicity associated with PQ dosing in a G6PD deficient strain of C3H mice was also observed after co-administration of PX. These data suggest that CYP 2D6 plays an integral role in the metabolic pathways necessary for PQ's efficacy and hemolytic toxicity. While the effects of MAO-A inhibition on toxicity remain unknown, metabolic compensation may account for increased efficacy as a result of decreased primaquine clearance.

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REPORTED ADVERSE EVENTS ASSOCIATED WITH ARTEMISININ COMBINATION THERAPIES (ACTS) IN NORTHERN GHANA

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Many African countries have adopted artemisinin derivative based combination therapy (ACT) as treatment for uncomplicated malaria, offering an opportunity to assess the safety of these drugs when in real life setting. Knowledge of side effects of these drugs is important for improved management of malaria. This study was conducted to document adverse events associated with Artesunate Amodiaquine (ASAQ), Arthemether Lumefantrine(AL) and Dihydroartemisin Piperaquine (DHP) through comprehensive pharmacovigilance in the Kassena Nankana districts of Northern Ghana. As part of INDEPTH Effectiveness and Safety studies, a cohort event monitoring study was conducted at selected public and private health facilities after administration of artemisinin combination therapy to participants to treat uncomplicated malaria during visits to the hospital. Participants were recruited if they were prescribed an ACT. Each participant was followed up between the 3rd and 7th day after enrolment to document adverse events. A total of 4951 participants with uncomplicated malaria prescribed ASAQ, AL and DHP were recruited across all age groups from August 2010 to June 2011. Out of the 4951 participants recruited, 26.0 % reported at least one adverse event; none had a serious adverse event. Of the 1288 participants reporting an adverse event 78.0%, 19.4% and 2.6% took ASAQ, AL and DHP respectively. 27.1% of females reported an adverse event compared to 19.8% of males recruited. Participants in the 15-49 years age group reported 43.3% of adverse events. The most reported adverse events were dizziness (24.2%) and weakness (23.5%) and these were more associated with ASAQ.The unadjusted odds ratio for participants who took AL are 2.3 (95% CI: 1.9-

2.8; p-value<0.001) times more likely to adhere to treatment compared to participants on ASAQ. Participants given DHP were also 10.7 (95% CI:4.4- 26.2; p-value<0.001) more likely to adhere to treatment compared to participants on ASAQ. 81.6% of those who had no adverse event adhered to treatment compared to those who had at least one adverse event, 18.4%. There were significantly more adverse events experienced by patients who took ASAQ compared to AL and DHP and this affected adherence to treatment.

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EVALUATION OF TWO QUALITY ASSURANCE APPROACHES FOR MALARIA RAPID DIAGNOSTIC TESTS IN PERIPHERAL HEALTH FACILITIES IN RURAL TANZANIA

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WHO recommends parasitological confirmation of malaria before treatment. Limited availability of microscopy in malaria endemic countries has resulted in increased use of antigen-detecting malaria rapid diagnostic tests (mRDTs), but appropriate quality assurance (QA) systems for mRDTs remain a concern. Tanzania has begun a national scale-up of mRDTs at all government health facilities. We evaluated mRDT performance in two districts of Tanzania with low malaria transmission using two QA methods: a) reference microscopy and b) detection of parasite DNA by real-time quantitative polymerase chain reaction (qPCR). Blood samples were collected from patients undergoing mRDT during two to three consecutive days each month in 12 health facilities between January and August 2010. Thick blood films were examined at the district headquarters and the Ifakara Health Institute (IHI) Bagamoyo Laboratory. A third blood film reader was consulted for discordant results. Molecular analysis involved extraction of parasite DNA from dried blood spots tested for presence of *Plasmodium falciparum* DNA with a pilot real-time assay targeting the tubulin gene. The assay was performed at IHI and about 40% of the DNA aliquots were sent to CDC for validation of IHI results. Samples from 1,837 patients were analyzed. Malaria positivity rates were 6.5%, 3.4%, and 2.7% for mRDT, qPCR, and microscopy, respectively. When qPCR was a gold-standard, mRDTs had higher sensitivity (68.6%; 95% CI: 55.0-79.7) than microscopy (53.7%; 95% CI: 38.7-68.0), but the difference was not significant. When microscopy was the gold standard, mRDT sensitivity was the highest (85.3%; 95% CI: 70.0-93.6). With qPCR as a gold standard, positive predictive values were significantly different between the two tests: microscopy vs qPCR-IHI (75.9%; 95% CI: 58.0-78.8), and mRDTs vs. qPCR-IHI (36.5%; 95% CI: 27.5-46.4). Higher inter-observer agreement (kappa=0.75) was seen amongst the microscopists. We identified many technical problems with qPCR analysis. QPCR may not be an appropriate QA tool to assess mRDT performance for routine care in this setting. A microscopy-based QA system may be a more suitable option.

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IMPACT OF INTENSIVE MALARIA MICROSCOPY TRAINING ON DISTRICT HOSPITALS LABORATORY STAFF IN TANZANIA

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Malaria microscopy training of laboratory staff responsible for definitive malaria identification, quantification, and speciation has a direct impact on achieving proper malaria diagnosis. We investigated the impact of a malaria diagnostics training program developed at the Malaria Diagnostics Center in Kisumu, Kenya on the overall understanding and performance of malaria microscopists in Tanzania. The objective of this analysis was to determine the optimal course syllabus and training duration necessary for strengthening clinic laboratory diagnosis of malaria in Tanzania. Pre- and post-course test results from 2009 to 2011 were compared against trainees'stratified laboratory training background. The time dedicated to performing microscopy practical sessions was recorded to determine if the optimal training time was enough to maximize accurate malaria diagnosis. For the 78 course participants tested it was observed that there is an improvement on the performance of laboratory in reaching a proper malaria diagnosis. Individual average scores for sensitivity ranged from 44.5% to 81.5% with an average improvement of 37% from pretest to post test. specificity test scores from 34.5% to 86% with an average improvement of 51.5%, written test scores from 53to 82.5% with an average improvement of 29.5% species identification from 40% to 71.5% with an average improvement of 31.5%, parasite detection from 64% to 79.5% with an average improvement of 15.5%, and parasite counting from 42.5% to 74.0% with an average improvement of 31.5%. There is a clear need for conducting regular and standardized malaria microscopy training for all laboratory staff dealing with malaria microscopy on a day-to-day basis. A two week training course can increase the level of malaria microscopy proficiency in Tanzania.

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THE ROLE OF BASELINE ASSESSMENTS ON MALARIA MICROSCOPY TOWARDS IMPROVING THE QUALITY OF MALARIA DIAGNOSTICS IN TANZANIA

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Well performed malaria microscopy has high sensitivity, can identify malaria species and quantitate parasitaemia which in turn will lead to appropriate clinical malaria management. Poor microscopic diagnosis of malaria results in both false positive and false negative results that directly result in poor clinical management and loss of physician confidence in laboratory results. We performed baseline assessments at 22 district hospital laboratories in three regions in Tanzania (Coastal, Kagera, and Zanzibar) to determine the status of staff experience, malaria diagnostic knowledge, the availability of quality equipment and supplies, quality of malaria smear staining, laboratory documentation procedures, supply chain management, and other parameters. Our analysis of the baseline assessments indicate a need for focused improvement in malaria smear preparation and staining procedures, distribution of high quality microscopes, use of high quality malaria smear staining reagents, use of standardized laboratory operating procedures, the development of a

robust supply chain management system, and implementation of QA/QC procedures. Areas that were performed well include laboratory registry documentation, generally consistent power supply, and patient safety procedures. The effectiveness of malaria microscopy training will be significantly augmented if the laboratory infrastructure enables microscopists to apply their training in routine practice. To this end there is an immediate need to improve the quality of laboratory equipment, supplies and improved standardized methodology to consistently prepare good quality stained malaria slides. The dual implementation of strengthened laboratory infrastructure and training will increase both malaria diagnostic capacity and competency that will directly lead to increasing accurate diagnosis. The duel focus on training and infrastructure strengthening will be the focus of ongoing WRAIR contributions to the PMI efforts in Tanzania and will directly improve the effectiveness of treatment and prevent over- or misuse of antimalarials.

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BASELINE ASSESSMENTS ON THE USE OF MALARIA RAPID DIAGNOSTIC TESTS (MRDT) IN HOSPITALS AND DISPENSARIES IN TANZANIA

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A key Tanzanian National Malaria Control Program (NMCP) objective is to increase the percentage of malaria microscopy and malaria rapid diagnostic test (mRDT) confirmed cases of malaria in public health facilities from 20% to 80% by 2012. Malaria diagnosis by microscopy requires well trained technicians and quality equipment, supplies and procedures. Increasingly Tanzania is relying on mRDTs for point-of-care malaria diagnosis in hospitals, health centres and peripheral dispensaries. Similar to microscopy, mRDTs can suffer from logistical supply chain problems, lack of quality assurance/quality control (QA/QC) procedures, and infrastructure deficiencies. In 20xx we conducted baseline assessments of healthcare workers' performance of mRDT in 6 district hospitals in the Coastal Region, plus 7 regional/district hospitals and 13 health centers/dispensaries in the Kagera Region. Parameters assessed included testing procedures and performance, supply chain management, QA/QC, staff training, documentation, and storage and waste management. Significantly 44% (7/16) of health facilities scored ≤60% for testing performance and only one of 16 health facilities achieved 90%. Our overall analysis of the baseline assessments indicate need for focused improvement in the support provided to testing staff, including job aids, timers and adequate ambient lighting; increased supervision of testing performance; increased availability of training; strengthened training in test interpretation; and the implementation of QA/QC procedures. Improving mRDT testing and supply management will directly lead to increasing accurate diagnosis to improve the effectiveness of treatment in Tanzania. Implementing these changes will be the focus of ongoing efforts to strengthen malaria diagnostic services in Tanzania.

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MISCLASSIFICATION OF *PLASMODIUM* SPECIES BY CONVENTIONAL MICROSCOPY

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Microscopic differentiation of *Plasmodium* species relies on morphological characteristics manifested in stained blood films. Proficiency levels of microscopists as well as morphological variations within and between *Plasmodium* species may lead to misclassifications. Ten- day microscopy workshops were conducted from 2009 to 2010. Proficiency of the participants was assessed at the start and the end of each workshop using mono infection slide sets of *P. falciparum*, *P. malariae*, *P. ovale* and *P. vivax.* Each slide with densities between 1,000 to 30,000 parasites/µL was examined for 5 minutes. Errors observed on each of the *Plasmodium* species were false negatives, positive results with no species indicated, inability to differentiate between *Plasmodium* species and reports of mixed infections. Pre- workshop misclassification of *P. falciparum* as positive was significantly higher ($p < 0.05$) than all other reported misclassifications except *P. malariae.* Misclassification of *P. malariae* as negative was significantly higher than all other reported misclassifications. Misclassification of *P. ovale* and P. *vivax* as mixed infections was significantly lower than all the reported misclassifications. Post workshop misclassification of *P. falciparum* as mixed infections was significantly higher than all other reported misclassifications and there was no clear misclassification of *P. malariae*. *P. ovale* was highly misclassified as *P. vivax* and *P. vivax* equally misclassified as *P. ovale.* Microscopy workshops can minimize observed errors and improve reliability of both clinical and epidemiological data. Confirmation of results by expert microscopy in addition to molecular characterization of species is highly recommended.

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MALARIA MICROSCOPY QUALITY ASSURANCE USING A SMALL NUMBER OF SLIDES

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WHO's "Universal Access to Malaria Diagnostic Testing, an Operational Manual" released in 2011 recommends "cross-checking of 10 to 20 slides if time (during a supervisory visit) allows." Weak infrastructure in most Sub-Saharan African countries' will hinder adherence to the previous WHO's Malaria Microscopy Quality Assurance (MMQA) protocol released in 2009 of selecting five negatives and five weak positives per lab and per month and sending them to a certified microscopist at a referral lab for cross-checking. For health facilities in many African countries, there are not enough slides available and no fuel or transportation to reference labs. Even if slides can be sent, there are not enough skilled microscopists at the reference labs to read all the slides received. The backlog of unread slides contributes to long delays feedback for their work. The Improving Malaria Diagnostic (IMaD) project tested if slide cross-checking during Outreach Training and Support Supervision (OTSS) visits could be used to identify underperforming labs and engage in on-the-spot problem-solving to address any deficiencies in slide preparation, staining or examination. As lab supervisors stayed in the lab for only one or two days, the number of slides cross-checked during the supervisory visit was on average 9.15 (standard deviation 1.96 slides) 76% of facility visits saving slides of QA. In Benin, 78% of laboratories visited for OTSS cumulated 12-20 slides in two consecutive visits. A sample size of 12 slides per health facility identified facilities under the decision rule for 90% parasite detection, and/or below average parasite detection. If OTSS is done quarterly, the minimum annual

aggregate number of slides would be 24, out of a target number of 40 slides (ten/visit), as opposed to 120 done by following the standard WHO MMQA 2009 protocol. Forty slides cumulated in a year selected randomly as per the LQAS stratified random sampling -with 50% slide positivity ratio- give a sufficiently precise estimate of parasite detection at laboratory level, allowing calculation of the % of laboratories attaining 90% agreement, and aggregate measurements at health zone level to focus MMQA efforts where they are needed the most. Resource-poor countries would be better served by considering a smaller sample size for MMQA selected with LQAS as opposed to not doing MMQA at all or doing MMQA in a way that fails to deliver feedback to participating labs.

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COMPARATIVE LABORATORY-BASED EVALUATION OF DIAGNOSTIC TESTS FOR G6PD

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Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common human enzyme deficiency in the world, affecting over 400 million people worldwide. It is characterized by abnormally low levels of G6PD, an enzyme involved in red blood cell metabolism. Individuals with diminished G6PD activity are susceptible to cellular oxidative damage, and can exhibit symptoms including hemolytic anemia and jaundice in response to a number of causes, most commonly infection or exposure to certain medications. In particular, treatment with anti-malarial drugs such as those in the 8-aminoquinolone group (e.g. Primaquine, Pamaquine and Tafenoquine) can cause acute hemolysis in people with G6PD deficiency. Because of this risk it is imperative to identify individuals with G6PD deficiency prior to administering these anti-malarial agents. As such, there is a need for a test that is appropriate for G6PD deficiency screening in areas of the developing world where malarial treatments are frequently administered. To explore the suitability of G6PD tests for use in conjunction with malarial management we conducted a laboratory-based evaluation to assess the performance and operational characteristics of several existing G6PD diagnostic tests. Tests evaluated included both qualitative and quantitative tests, utilizing a variety of test formats (fluorescent spot test, rapid point-of-care tests, dye reduction tests, and spectrophotometry-based tests). Our findings indicate that most of the currently available diagnostic tests for G6PD appear to have technical or operational shortcomings that may limit their applicability to low-resource malaria management settings. Further adaptation and/or modification of existing tests or development of new tests to better meet the needs of clinicians and laboratory staff involved in malaria-case management in the developing world may be needed. We present data from this evaluation and critical design inputs to guide development of new diagnostic tests for G6PD testing.

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DEVELOPMENT OF A READY-TO-USE GELIFIED REAL-TIME PCR ASSAY FOR SIMULTANEOUS SPECIFIC IDENTIFICATION OF *PLASMODIUM FALCIPARUM*, *P. MALARIAE*, *P. VIVAX*, AND *P. OVALE* IN CLINICAL SAMPLES

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The use of PCR for identification *Plasmodium* spp. represents an attractive alternative for diagnosis of malaria. Some robust PCR techniques exist for this purpose, but they still based on complex procedures. This is not only time consuming, but increases the cost of PCR applications limiting its usefulness for laboratories in developing countries. Nevertheless, this can be drastically changed with PCR techniques designed to be executed under minimal quality control standards. We selected a previously published TaqMan assay and converted into a gelified format for robust, specific and simplified multiplex identification of *P. falciparum, P. malariae*, *P. vivax,* and *P. ovale*. Gelification consists in a process where the components of enzymatic reactions are stabilized by addition of different agents. In order to execute the procedure the laboratorian simply needs to add water and the DNA sample to the reaction tubes coated with all chemicals required for PCR amplification. Next, the vessels containing the re-solubilized mixture and the template are inserted into the real-time PCR thermal cycler for DNA amplification. The preliminary evaluation assay's liquid format indicated that it was very specific compared to the nested PCR, since it did not produce any cross-amplification with samples containing other *Plasmodium* species such as *P. cynomolgi*, *P. hylobati*, *P. inui* and *P. knowlesi* (N=14); nested PCR primers for *P. vivax* cross-amplified *P. cynomolgi* (N=4). No false negative or false positive results were verified when this assay was compared to the nested PCR using approximately 100 blood specimens sent to CDC for confirmatory diagnosis of malaria. This evaluation showed that the gelified assay had more efficient amplification profiles in addition to being simple to execute and providing results within 2 hours, including preparation time. Also, the gelified format of the assay was stable for 7 days at room temperature and for 2 months at 4 C. We believe that the gelified assay format can streamline the use of real-time PCR for confirmatory diagnosis of malaria.

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A METHOD FOR IMPROVING THICK BLOOD FILM SLIDE ADHERENCE FOR MALARIA DIAGNOSIS

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The gold standard for malaria diagnosis remains the examination of thick and thin blood films. The thick film contains a greater amount of blood than the thin film and thus provides the greatest sensitivity for screening. Unfortunately, the larger quantity of blood may not adequately adhere to the thick film and some or all of the droplet may separate from the slide during staining. The possibility of sample loss has led to recommendations that thick films be allowed to dry from 3 hours to overnight to improve the blood droplet adherence to the slide. This delay in preparation of the thick film can delay diagnosis and treatment decisions if parasitemia is not evident on the thin film. Here we describe a simple and inexpensive 'scratch method' for improving thick blood film adherence, ameliorating the need for extended drying times. Standardized blood droplets (35 microliters) from twenty-six previously examined EDTA whole blood specimens (22 positive and 4 negative) were used to prepare Giemsa-stained thick films either by a traditional or scratch method. By the traditional method, the droplet was gently spread to an approximate nickel-sized area (22 mm diameter) on the slide using the edge of a second glass slide. Using the scratch method, the droplet was smeared while forcefully grinding or 'scratching' it into the slide with the point of a second glass slide. All slides were dried for 1 hour in a laminar flow hood and Giemsa-stained using established protocols. Slides were then examined in a blinded manner for parasite identification, determination of percent parasitemia, and degree of blood droplet adherence by 4 independent trained examiners. There was no difference in detection of parasites or parasite morphology between the two methods, but blood droplet adherence was significantly improved by the scratch method. The scratch method is a simple and effective way to improve thick film adherence and thus facilitate rapid screening. This method does not require additional equipment or significant changes in sample preparation methods.

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ANALYSIS OF DISCORDANT RESULTS BETWEEN MALARIA RAPID DIAGNOSIS TESTS (TDRS) AND MICROSCOPY

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The thick smear, a recommended blood test when diagnosing malaria, is a technique with some limits and that still is out of reach for people living in remote zones from endemic regions. Perfecting TDRs, means an easy and fast technique for malaria diagnosis, could, however help to make up these shortcomings. Nevertheless, their efficacy and effectiveness should be assessed in order to determine their performance. During a study conducted in Gabon, an HRP2 TDR (Aco® and the pLDH TDR Optimal-IT, 15% of discrepancies were found between TDRs and the thick smear. Our study aimed at analyzing these discordant results using the nested PCR, for amplification of the gene representing the small under-unit of the ARN 18S. Out of the 415 analyzed samples (307 differences and 108 correspondences), 28,4% (38/171) were positive with the PCR. The *Plasmodium falciparum* infection was detected in 22,2% (38/171) of the positive samples with the Acon test, corresponding to 77,8% of false positive results and to more than 80% of bands with low intensity. The proportion of false negatives was 25,6%. The proportion of false positive with the test Optimal-IT (78,2%), was due to false detection of non *falciparum* species ; that of the false negatives was lower (33%). Sensitivity, specificity and negative predictive value of both TDRs with the thick smear corrected by the PCR considered as the reference exceeded 90%, except for the detection of the non *falciparum* species with Optimal-IT. Acon® and Optimal-IT remain of good interest for the biological diagnosis of malaria in areas where thick smears and well trained microscopists are not available.

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PLASMODIUM FALCIPARUM HISTIDINE-RICH PROTEIN 2 IN HUMAN MALARIA: DYNAMICS AND DETECTION BY RAPID DIAGNOSTIC TESTS

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Rapid diagnostic tests (RDTs) based on *Plasmodium falciparum* histidine-rich protein 2 (*Pf*HRP2) are popular for diagnosis of this most virulent malaria infection. However, concerns have been raised about the longevity of the *Pf*HRP2 antigenemia following curative treatment in endemic regions. It is also unclear how early in an infection the *Pf*HRP2-detecting RDTs become positive. These are important questions for the widespread use of malaria RDTs in diagnosing malaria in clinical settings. We developed a mathematical model of *Pf*HRP2 production and decay to mimic the kinetics of antigenemia during human infections. Fitting this *Pf*HRP2 dynamics model to data from human infection studies in malaria naïve hosts allowed the amount of *Pf*HRP2 produced per parasite to be estimated at g per replication cycle. The limit of detection for four commercial malaria RDTs was assessed in the laboratory; the minimum detection thresholds were between 6.9 and 27.8 ng/mL. The low detection threshold and long-half life of *Pf*HRP2 is predicted to cause the RDTs to remain positive for at least 7 days after treatment. However the actual duration of positive test results following curative treatment is heavily dependent on the duration and density of parasitemia prior to treatment. Combining the model output and RDT sensitivity data, it is predicted that good quality *Pf*HRP2-based RDTs should be able to detect parasites on the first day of symptoms in malaria naïve individuals, and that as few as 8 parasites/µL may be required to maintain a positive RDT in a chronic *P. falciparum* infection. The impact that host anti-*Pf*HRP2 antibodies may have on RDT positivity in endemic regions is currently being investigated.

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MOLECULAR DIAGNOSIS OF MALARIA BY PHOTO-INDUCED ELECTRON TRANSFER FLUOROGENIC PRIMERS (PET-PCR)

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Malaria control and elimination programs require the use of diagnostic tools that are sensitive, cost effective and able to detect multiple species simultaneously in a simple and accurate manner. The real-time PCR methods are particularly robust for large scale screening and there is scope for improving these methods for field applications. Here, we have designed novel self-quenching real-time PCR primers for the detection of *Plasmodium* spp. and *P. falciparum*. This PCR assay uses the photo-induced electron transfer (PET) chemistry and therefore does not require internal probes, which are usually very expensive or intercalating dyes, which are often non-specific. A total of 115 clinical samples consisting of different malaria species and some mixed infections (9 malaria negative samples, 81 *P. falciparum*, 9 *P. vivax*, 1 *P. malariae*, 9 *P. ovale, 2 P. falciparum/P. malariae, 1 P. vivax/P. ovale, 2 P. falciparum/P. ovale* mixed infections and 1 *P. knowlesi*) were used to test the utility of the novel PET-PCR primers in diagnosis of clinical samples. The sensitivity and specificity was calculated using a nested PCR as a gold standard. Both primer sets showed 100% sensitivity and specificity. This malaria PET-PCR method can detect parasite densities as low as 10 parasites/µL of both *Plasmodium* spp. and *P. falciparum*. In addition, the reaction can be duplexed to detect both *Plasmodium* spp. and *P. falciparum* in a single reaction. Further validation of this technique in field settings will help to assess its utility for large scale screening for malaria parasitemia, potentially important for control and elimination programs.

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DEVELOPMENT OF A FLUORESCENCE IMMUNOASSAY FOR SEMI-QUANTITATIVE OF THE DIAGNOSIS MALARIA: *PLASMODIUM FALCIPARUM AND PLASMODIUM VIVAX*

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The rapid and accurate diagnosis of malaria is key to the central to clinical management and the prevention of drug-overuse, which may lead to resistance development, toxicity and economic loss. So far, microscopy of Giemsa-stained thin or thick blood smears is the gold standard. Rapid diagnosis tests provide an alternative, although they cost more and give qualitative instead of quantitative results. A fluorescence (FL) dye-incorporated immunochromatographic assay (ICA) might offer a higher sensitivity than rapid device which can be used at the point of care testing(POCT). The fluorescence immunoassay was employed to detect and semi-quantitative *Plasmodium falciparum* (P.f) and *Plasmodium viva x*(P.v) malaria-infected whole blood. It consisted of a FL antibody detector buffer, a test strip housed in a disposable cartridge, and a laser fluorescence scanner. The whole blood mixed with detector, loaded onto a cartridge, incubated 10 minutes, and the semi-quantitative of P.f and P.v malaria parasites were measured in a fluorescence scanner. The comparability of the new method was examined with microscopy check and rapid device malaria diagnosis. By microscopy, *Plasmodium* was detected successfully in all 81 clinically suspected malaria patients, including 59 individuals with low parasitemia (1-100 parasites/µl) and 22 individuals with middle parasitemia (101-500 parasites/µl). At low parasitemia (1-100 parasites/µl), sensitivities for FL-ICA and microscopy check were 80% and 100%, respectively. The accuracy of semi-quantitative was 90%. At middle parasitemia (1-100 parasites/µl), sensitivities for FL-ICA and microscopy check were 95% and 100%, respectively. In conclusion, while the approximate accuracy of semi-

quantitative test was 95%, the new fluorescence immunoassay may be used as a POCT diagnostic tool and has potentials as a fast, accurate, reliable, easy, and suitable tool for the semi-quantitation analysis for P.f and P.v malaria diagnosis.

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MODELING HEALTH SYSTEMS BARRIERS TO SUCCESSFUL MALARIA MANAGEMENT

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A functioning and efficient health system is required to maintain reductions in malaria disease and transmission. Few models demonstrate how to deliver a proven intervention most effectively through an existing system. The "systems effectiveness framework" has previously been used to describe how a cascade of interacting health systems barriers may sequentially reduce the effectiveness of treatment interventions. We contrasted this approach with a decision analysis model of malaria treatment in the public sector. A common set of parameters for malaria management in Africa including access to care, diagnosis and treatment were obtained from the literature. The decision analysis model more accurately reflected reported levels of appropriate management of fever (malarial and non-malarial) in the public sector (>50% attendees) compared with a systems effectiveness approach (<15%). Modeling increases in availability and usage of rapid diagnostic tests (RDTs) improved overall management of fever (upto 80% attendees) and reduced over-treatment of non-malarial fevers with anti-malarials (<12%), but had less impact on the proportion of malaria cases treated (<57%). In contrast, reducing stockouts of first-line anti-malarials had a substantial impact on the proportion of malaria cases treated (68%)even without increased RDT use. Improving adherence to test results was not predicted to substantially improve appropriate treatment rates for malaria since the risk of under-treatment is low, and baseline utilisation of RDTs was assumed to be only 40% as per the literature. Under conditions of perfect availability and use of RDTs, test adherence and drug availability, appropriate treatment rates were predicted to rise to 95%. Simple decision analysis models can provide insight into which aspects of delivering care are most likely to impact on care quality and treatment effectiveness, and at different transmission intensities. Further work into the amenability of health systems to change is required to explore the most cost-effective targets in expanding the delivery of anti-malarials.

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RAPID DIAGNOSTIC TEST PERFORMANCE IN THE SETTING OF DIFFERING TRANSMISSION INTENSITIES: THE MALAWI ICEMR EXPERIENCE

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In Malawi, like many malaria-endemic countries, rapid diagnostic tests (RDTs) are used to confirm the diagnosis of malaria because access to microscopy is limited. Much attention has been given to the sensitivity of RDTs, but their positive predictive value has not been explored, especially in areas such as Malawi, where malaria continues to be endemic. RDTs may be positive for weeks following successful treatment. In cases of false positive RDTs evaluation and treatment of alternative diagnoses might be neglected because of the presumed malaria diagnosis. Through the Malawi International Center of Excellence in Malaria Research, we are conducting surveillance for malaria in three transmission settings using RDTs (Paracheck®), microscopy and molecular detection of malaria infection. Among all surveillance sites during the rainy season, 25-30% of people with symptoms compatible with malaria had had a positive RDT. We conducted a preliminary evaluation comparing RDT results to expert microscopy. Overall, the positive predictive value (PPV) of a RDT compared to microscopy was 76.1%. The RDT PPV was inversely related to transmission intensity. In the moderate transmission intensity regions of the rural highlands and urban highlands, PPV was 91.7% and 72.3%, while in the lowland area with intense malaria transmission the PPV was 66.7%. In the areas of moderate transmission, the PPV was higher in adults compared to children under five years of age (97.9% vs. 84.2% and 80.5% vs. 55.6% in the highlands and urban setting respectively). In contrast, in the most intense transmission region, PPV was slightly lower in adults compared to children (64.2% vs. 73.5%). Microscopy is being conducted on additional slides collected from patients with positive RDTs in both the rainy and dry seasons. Sensitivity and specificity compared to molecular diagnosis will also be reported. The rate of false positive RDTs is high and is related to transmission intensity. This raises the concern that alternative causes of illness will not be pursued in patients with a positive RDT.

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IMPROVING ANTIMALARIAL DRUG RESISTANCE SURVEILLANCE THROUGH EXTERNAL QUALITY ASSESSMENT AND PROFICIENCY TESTING PROGRAMS

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Comprehensive antimalarial drug resistance surveillance includes measures of recurrent parasitemia, *in vitro* drug susceptibility, drug concentrations to differentiate true resistance from inadequate drug exposure, and genotyping to distinguish recrudescence from re-infection. External quality assessment and proficiency testing (PT) are key components of quality control for laboratory procedures. The goals of the WWARN QA/QC program are to improve and maintain high-quality antimalarial drug analysis, *in vitro* drug susceptibility testing, and genotyping, thereby improving the quality of data. The program includes PT for pharmacology laboratories, a reference material program that provides pure antimalarial drug standards, metabolites and internal standards for pharmacology and in-vitro laboratories, and a molecular PT program. WWARN is developing international networks of laboratories doing antimalarial drug testing and genotyping. The reference material program distributed accurately weighed quantities of antimalarial drug standards, metabolites and internal standards to 25 laboratories. The pharmacology PT program sent samples to 8 laboratories in 4 rounds of PT. The molecular PT program includes biannual PT to differentiate parasite recrudescence from re-infection for 8 laboratories in 6 countries. Data will be presented showing how participating laboratories have improved significantly over subsequent rounds of PT. The benefits of the reference material program include cost savings for the laboratories and provision of a uniform standard of material, reducing inter-laboratory variability. Benefits of participating in the PT program include identification of technical difficulties encountered in the analysis of drug compounds and genotyping. Technical experts provide advice for correcting problems to improve performance in subsequent analysis, and ultimately improve the quality of drug resistance surveillance data and facilitate pooled analyses.

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PFMDR1 IS ASSOCIATED WITH RECRUDESCENCE AFTER TREATMENT WITH ARTMETHER-LUMEFANTRINE IN WESTERN KENYA

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Single Nucleotide Polymorphsms (SNPs) in PfMDR1 and PfCRT have been associated with *Plasmodium falciparum* resistance to drugs including chloroquine (CQ), amodiaquine (AQ), lumefantrine (LU) and mefloquine (MQ). Artmether-Lumefantrine (AL) is currently the first line antimalarial used in Kenya with artesunate-amodiaquine (ASAQ) and dihydroartemisin piperaquine (DHA-PPQ) being readily available from private retailers. During an open-label randomized clinical study evaluating the efficacy of AL in Ombeyi, a malarial endemic district in Western-Kenya, we investigated the role of PfMDR1 and PfCRT in modulating tolerance to artemisinin partner drugs. All recurrent samples were genotyped for MSP1, MSP2 and GLURP at day 0 and day of recurrence. Additionally all samples were assayed for SNPs in PfMDR1 codon 86 and PfCRT codon 72-76 as well as copy numbers in PfMDR1. All day 0 samples were assayed for drug susceptibility using the SYBR Green method. Among the 454 subjects enrolled in the study, there were 162 recurrent cases of which 134 were reinfections while 17 were recrudescences and 11 undetermined. PfMDR1 N86 was significantly associated with recrudescence compared to both day 0 and reinfection. There was no significant association between PfCRT and recurrent infections and amplification of PfMDR1 gene was not observed. Significant positive correlation was observed between LU and MQ ($r=0.5$, $r^2= 0.27$, $p<0.05$). This data demonstrates an association between PfMDR1 N86 and recrudescence after treatment with AL in Western Kenya. Co-resistance between LU and MQ indicates that LU pressure may lead to MQ resistance, an important prophylaxis for malaria naïve visitors to Kenya.

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ANTI *PLASMODIUM FALCIPARUM* MO15-RELATED PROTEIN KINASE *(PF*MRK) AND *P. FALCIPARUM* PROTEIN KINASE 5 (PFPK5) ACTIVITIES OF NATURAL PRODUCTS FROM PLANTS

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There is urgent need to develop new chemotherapeutic anti-*Plasmodium falciparum* to replenish loses to resistance. Natural products, including flavonoids and quinones, currently being explored as anticancer agents inhibiting cyclin dependent kinases (CDKs) are also antiplasmodial, but information on their mechanism of action is scanty. This study assessed the *in-vitro* flavonoids and quinones inhibition of *Plasmodium falciparum MO15-related protein kinase (Pf*mrk) and *Plasmodium falciparum* protein kinase 5 (PfPK5) using luciferase-coupled kinase assay. These compounds were obtained from six East African plants. Compounds coded as BA-4E, 0.26μM and BA-6S 0.22μM were the most active against Pfmrk and PfPK5 respectively while BA-6U (0.4μM) and BA-4C (1.03μM) showed specificity against Pfmrk. Flavonoids of the subclass flavanones were the most active compounds. Flavanones having two prenyl substituents (diprenylated compounds) on ring B with a hydroxyl or methoxy group at position 4´ had highest activity regardless of these groups' position. These findings suggest that inhibition of *Pf*mrk and/or PfPK5 may be among ways that flavonoids inhibit *Pf* replication.

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EFFICACY OF ARTEMETHER-LUMEFANTRINE FOR THE TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* INFECTION IN TANZANIA

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Tanzania mainland through the ministry of health and social welfare (MoHSW) introduced artemisinin combination therapy (ACT) with artemether-lumefantrine (ALu) as first line treatment policy for uncomplicated *falciparum* malaria since 2006. Despite good profile of ALu on malaria control, there is a threat of ACT drug resistance. Due to recent report on the emerging drug resistance to ACT along the Thai-Cambodia border it is critical to our region to monitor the spread of drug resistance to ACT. Five years after countrywide policy implementation in Tanzania there has been no systematically designed and implemented efficacy monitoring study to assess ACT. In 2011 Ifakara Health Institute in collaboration with other country based researchers conducted a round of assessment of anti-malaria through 4 out of 8 invivo MOHSW monitoring sentinel sites that recorded good performance of both ALu and amodiaquine-artesunate (ASAQ). Because coverage of ACT profile across the remaining 4 sentinel sites is envisaged to portray a countrywide ACT performance, beginning in May 2012 another round of efficacy monitoring is planned to be implemented. We therefore set up to conduct an invivo monitoring study at four sentinel representative National Malaria Control Programme (NMCP)'s sites in May-September 2012 to assess efficacy of ALu. This study will be conducted at sites in Kyela, Masasi, Chamwino and Butimba in mainland Tanzania. Participants are febrile patients aged 6 months-10 years presenting at the health facility to be followed up during 28 days. It is intended to elicit treatment performance using 2010 WHO protocol. Results of this study will be out by the time of American Society of Tropical Medicine and Hygiene conference in November 2012. We will elucidate the occurrence of recrudescence by PCR using msp1 and glurp. Results from this study will be used to assist the MoHSW assess the current national treatment guidelines for uncomplicated *falciparum* malaria and update the global initiatives to containment of ACT drug resistance.

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CHLOROQUINE-RESISTANT *PLASMODIUM FALCIPARUM* MALARIA IN TRAVELERS FROM HAITI AFTER THE 2010 EARTHQUAKE

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Chloroquine (CQ) associated with primaquine is recommended as first-line treatment for uncomplicated malaria in Haiti. CQ *in vitro* and molecular surveillance data collected over the past two decades suggest continued *Plasmodium falciparum* sensitivity. However, a 2006-2007 study showed around 6% (5/79) of *P. falciparum* isolates had the CQ resistance-associated pfcrt76T genotype. The January 2010 earthquake and flooding following Hurricane "Tomas" later in the year may have created conditions for increased malaria infections. We have investigated the CQ sensitivity of *P. falciparum* parasites isolated from travellers recently returned from Haiti to France and Canada, using genotypic and phenotypic methods. Retrospective data was collected from the French National Malaria Reference Centre (CNR) and the Public Health Ontario, 1988-2010 and 2007-2010, respectively. The definition of an infection probably acquired in Haiti was recent travel to the country prior to diagnosis with *P. falciparum* positive thin and thick blood smear. Basic demographic and epidemiologic data, clinic and parasitological information, treatment, history of travel and malaria infection were collected systematically. Prior to the earthquake, all isolates (n=29) had the wild-type pfcrtK76 allele, analysed by PCR-RFLP. The mean of the 50% growth inhibition (IC_{50}) of CQ of the isolates (n=24) was 27nM (95% confidence interval[CI], 23 to 31). After the earthquake, two of ten isolates showed CQ resistance *in vitro* after culture adaptation. Both isolates had high CQ IC50 (506nM and 708nM) and high CQ IC50 isolate:Pf3D7 (CQ susceptible clone) ratio (20 and 27). Resistance was confirmed by the molecular analysis demonstrating the presence of the CQ-resistant associated pfcrt76T allele (mixed K+T) only in these two isolates. Our data confirm the presence of CQ-resistant strains in Haiti. They highlight the importance to implement a therapeutic efficacy study for assessing *in vivo* CQ-sensitivity, essential for informing rational control strategies and guiding prophylaxis recommendations in Haiti.

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RETURN OF CHLOROQUINE SUSCEPTIBILITY OF *PLASMODIUM FALCIPARUM* STRAINS IN TRAVELERS RETURNING FROM WEST AFRICA

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Following WHO recommendations, most African countries have discontinued chloroquine (CQ), and now promote artemisinin-based combination therapy (ACT), as the first-line treatment for uncomplicated malaria. The policy changed in West Africa during the last decade (2002 in Cameroon; 2003 in Senegal and Cote d'Ivoire; 2004 in Mali). The aim of this study was to describe the evolution of CQ resistance in West Africa, through travelers returning from this region. The study was conducted by the Malaria National Reference Centre, France. The database collated *in vitro* response of reference and clinical isolates for CQ and the pfcrt K76 molecular marker for CQ susceptible Pf malaria. As a proxy of drug pressure, CQ intake for children under five years of age with fever was extracted from the Demographic Health Surveys (DHS) and Multiple Indicator Cluster Surveys (MICS). Logistic regression models were used to detect trends in the susceptible isolates proportions. From 2000 to 2011, around 700 isolates were genotyped for each country. The frequency of the pfcrt76 wild-type significantly increased for Cameroon (CM) (from 10% to 41%, Slope=0.09, p<10-3), Cote d'Ivoire (CI) (from 37% to 63%, Slope = 0.14, p<10-3), and Senegal (SN) (from 22% to 53%, Slope=0.17, p<10-3). The mean of the 50% growth inhibition (IC_{50}) of CQ decreased from 314nM (95% confidence interval, 102-526) to 101nM (71-131) in CM, from 109nM (70-148) to 47nM (28-66) in CI and from 144nM (91-196) to 75nM (36-115) in SN. Meanwhile, CQ use among children with fever significantly decreased during this period. An increase of CQ susceptibility following official withdrawal is observed in travelers returning from Cameroon, Cote d'Ivoire and Senegal. The length of time between policy changes and their subsequent implementation, as well as the cross resistance between antimalarial drugs, may affect the time for a significant recovery of CQ sensitivity. This information should be compared to country level CQ efficacy data.

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TRAVELER'S SURVEILLANCE: A TOOL FOR DETECTING EMERGENCE OF ANTIMALARIAL DRUG RESISTANCE IN ENDEMIC COUNTRIES

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There is growing concern about the emergence of resistance in Southeast-Asia to artemisinin-based combination therapy (ACT), the first-line treatment for malaria. In the time since the widespread adoption of ACTs, a decrease in the systematic surveillance of antimalarial drug resistance has been observed in many endemic countries. Furthermore, high levels of host immunity complicate the identification of treatment failures associated with resistance. The aim of this project was to validate the use of travelers returning from Africa with malaria as an additional surveillance system for the emergence of drug resistance. We compared data collected between 1998-2011, from the French Malaria Reference Centre for traveler's data versus field data from the literature and within the WWARN database. We compared temporal trends of the proportion of wildtype-genotype isolates for CRT76 and DHFR108 molecular markers, as well as the *in vitro* response to chloroquine (CQ) of isolates using generalized linear models. Three countries were selected for the analysis: Senegal (SN), Mali (ML) and Cameroon (CM) based on a required sample size of 600 isolates per group. For CRT76, no significant (NS) difference is shown between travelers and field studies in CM (slopeβ1=0.03, slopeβ2=0.03, respectively, p=NS), SN (β1=0.17, β2=0.21, respectively, p=NS) and ML (β1=-0.19, β2=-0.17, respectively, p=NS). These results are supported by *in vitro* analysis in SN (β1=-0.03, β2=-0.05, respectively, p=NS). An increase of CQ-sensitive isolates is observed, except for ML where only data up to 2004 was included. For DHFR108, no significant difference is shown between travelers and field studies in CM (β1=-0.24, β2=-0.10, respectively, p=NS), ML (β1=-0.17, β2=-0.11, respectively, p=NS) and SN (β1=-0.09, β2=-0.06, respectively, p=NS). A decrease of wildtype-genotype isolates is observed. Our results show similar trends in resistance

between travelers and field studies. This work highlights the value of an international traveler's database to assess and monitor the emergence of drug resistance in endemic areas where information is limited.

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MULTIPLE INSECTICIDE RESISTANCE IN WESTERN KENYA: IMPEDIMENT TO INSECTICIDE-BASED MALARIA VECTOR CONTROL PROGRAMS IN KENYA

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Indoor Residual Spraying (IRS) and long-lasting insecticidal nets (LLINs) have been extensively used for malaria prevention and control in Kenya. However, the development of resistance by mosquitoes to recommended insecticides for IRS and/or ITNs/LLINs would affect insecticide-based malaria vector control. We assessed the effect of extensive use of IRS and LLINs on development of resistance in *Anopheles gambiae* from western Kenya. Wall bioassays were performed on artificial walls and filter papers sprayed with ICON and deltamethrin using mosquitoes collected from different sites from western Kenya and Kisumu strain as a control. Net cone bioassays were also performed on nets collected from the fields using mosquitoes from two sites and Kisumu susceptible strain as a control. Chemical analysis of the netting material was also done using HPLC to determine the concentration of insecticides on the net. Kisumu strain was susceptible to all the insecticides with 100% mortality. Mosquitoes from Chulaimbo, Ahero, chulaimbo, Emakakha and Kisian shows susceptibility to both deltamethrin and ICON with the mortality rates ranging between 80% - 85% but mosquitoes from Bungoma and Emutete shows resistance to both ICON and deltamethrin with mortality rates ranging from 69%- 74%. Sprayed artificial walls shows lower mortality rates compare to sprayed filter papers. ICON had high mortality rates on the mosquitoes compared to Deltamethrin. Mosquitoes from Bungoma and Emutete showed resistance in Net bioassays with the mortality rates ranging between 60% -75%, but the control strain was highly susceptible to the nets with 100% mortality. HPLC results indicated that the nets still had a high concentration if insecticides ranging from 0.06 wt% - 0.19 wt%, the positive control net had the concentration of 0.14 wt%. The observed resistance to insecticides used for IRS and LLINs in *An. gambiae* Populations from western Kenya could affect the malaria vector control programmes in Kenya; therefore there is need urgent implementation of resistance management strategies and intergrated vector control intervention.

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MECHANISM OF ARTELINIC ACID RESISTANCE IN *PLASMODIUM FALCIPARUM IN VITRO*

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The emergence of *Plasmodium falciparum* parasites with decreased *in vivo* sensitivity in several South East Asian countries has raised the urgent need to understand the underlining biological mechanism. We investigated the processes involved in the development of artelinic acid resistance using laboratory generated resistant *P. falciparum* lines *in vitro*. Our results demonstrate that resistance to artelinic acid has two major characteristics: 1) resistance affecting early asexual stage parasites demonstrated by the insensitivity of ring-stage parasites to the induction of dormancy, and a faster recovery from dormancy when it is induced with higher drug concentrations. 2) resistance of late stage parasites which allows continuous growth and multiplication of parasites under continued drug pressure. These results demonstrate that changes in the dormancy profile of parasites are part of the resistance phenotype and suggest that the development of artemisinin resistance may involve two steps. The molecular events important in each step are currently being investigated to determine whether full artemisinin resistance develops as a stepwise process or whether the two stages arise independently of each other.

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PHARMACODYNAMICS OF ARTEMISININ COMBINATIONS FOR DRUG SENSITIVE AND RESISTANT *PLASMODIUM BERGHEI IN VIVO*

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Artemisinin combination therapies (ACTs) combine a very potent, yet short-lived artemisinin (ART) derivative with a partner drug that usually has a longer half-life, yet established resistance in the field. This strategy enhances therapeutic efficacy and theoretically delays the emergence of ART resistant *Plasmodium falciparum*. Although ACTs are the first-line treatment globally, pharmacodynamic (PD) properties of the combinations are not well studied. In particular the impact of existing resistance to the partner drug on emerging resistance and reduced clinically efficacy is poorly understood. To examine the PD properties of ACTs we used drug sensitive and resistant *P. berghei* in standard mouse efficacy models. In these studies we examined the PD properties of artemether-lumefantrine (ATM-LMF), artesunate-mefloquine (AS-MFQ), AS-amodiaquine (AS-ADQ), dihydroartemisinin-piperaquine (DHA-PIP), and AS-pyronaridine (AS-PND). First we used drug-sensitive *P. berghei* and identified minimal curative drug concentrations that rapidly clear parasite infections, prevent parasite recrudescence, and are not antagonistic. Secondly we evaluated the impact of resistance to the following partner drugs by using the MFQ-resistant N/1100, ADQ- resistant NAM, and PND resistant-NPN-10 lines of *P. berghei*. The data obtained thus far demonstrate that partner drug resistance significantly erodes the PD properties of the ACTs in current clinical use. For example with MFQ resistance, the minimal effective regimen of AS-MFA for sensitive parasites was poorly efficacious versus MFQ resistant parasites.These data demonstrate the utility of the rodent model to estimate PD properties of ACT combinations and to determine the most effective ACT regimens to delay emergence of ART resistance.

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HIGH THROUGHPUT ANALYSIS OF *IN VITRO* ANTIMALARIAL SENSITIVITY DATA IN THE ERA OF ARTEMISININ COMBINATION THERAPY: THE WWARN *IN VITRO* ANALYSIS AND REPORT TOOL (IVART)

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In vitro assessment of antimalarial drug sensitivity remains an important tool in the era of artemisinin combination therapy, providing a way to assess parasite susceptibility to a range of drugs that is largely independent of clinical factors. In addition, investigation of molecular mechanisms of resistance via transfection depends absolutely on accurate and relevant *in vitro* phenotyping. WWARN and its collaborators have established a repository of raw, *in vitro* data derived from a wide range of locations and readout methods. Analysis of this large and varied dataset has now been undertaken using WWARN's *In Vitro* Analysis and Report Tool (IVART), an application that performs high throughput data analysis with calculation of standard IC50 parameters via non-linear regression. Here we describe the development and validation of IVART, and report the evidence base for its design features, including methods for curve fitting and quality assessment that, until now, have relied on expert opinion.

The data also reveal differences in efficacy between artemisinins and their partner drugs in several forms of assay readout, highlighting the importance of matching *in vitro* assay readouts to *in vivo* properties in areas of emerging drug resistance. Improvements in the standardization of *in vitro* assays are critically important and the development of a free, adapted software tool like IVART addresses the heterogeneity of analytical *in vitro* output. Such standardized *in vitro* outputs could play a major role in the validation of potential molecular markers of resistance to antimalarials including artemisinin.

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TRENDS OF THE FREQUENCY OF *PLASMODIUM FALCIPARUM* DRUG-RESISTANCE MOLECULAR MARKERS IN ISOLATES FROM PREGNANT WOMEN SIX YEARS AFTER INTRODUCTION OF INTERMITTENT PREVENTIVE TREATMENT WITH SULFADOXINE-PYRIMETHAMINE (IPTP-SP) IN GABON

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Following WHO recommendations for malaria control, gabonese Ministry of Health adopted ACTs, insecticide-treated nets and intermittent preventive treatment with sulfadoxine-pyrimethamine (IPTp-SP) in 2003. Prevalence of triple dhfr and quintuple *dhfr/dhps* mutation were respectively of 86% and 22% in 2005. Six years after their implementation, the frequency of *dhfr* and *dhps* point mutations was assessed in *Plasmodium falciparum* isolates from Gabonese pregnant women according to the number of SP doses. Polymorphic codons of *dhfr* gene (51, 59, 108 and 164) and *dhps* gene (437, 540 and 581) were analysed using PCR-restriction fragment length polymorphism. Blood samples from 89 women were analyzed, 35 received 2 doses, 16 received 3 doses and 18 none dose of SP. Among patients with 3 SP doses, 11 had submicroscopic infection. None sample had a quadruple *dhfr* mutation but the frequency of triple mutation *(51-59-108)* was 98%. All parasites carried a wild-type allele at codon 164. The same was true for the codon 581 of *dhps* gene. These preliminary data indicate an increase in the frequency of multiple resistance markers to SP independently of the number of doses received during pregnancy. There is an urgent need to assess the *in vitro* susceptibility of *P. falciparum* isolates to SP, to study other factors associated with the presence of SP resistant parasites and to evaluate an alternative drug for IPTp for pregnant women.

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STUDIES ON AP2 ADAPTOR μ-CHAIN, A NEW CANDIDATE MOLECULAR MARKER FOR ARTEMISININ RESISTANCE IN *PLASMODIUM FALCIPARUM*

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There is evidence of reduced susceptibility of the malaria parasite *Plasmodium falciparum* to artemisinin derivatives, expressed by delayed parasite clearance times *in vivo*. If artemisinin resistance spreads, it would threaten global malaria control. We lack validated molecular markers for monitoring these phenotypes. Using whole genome sequencing in the rodent malaria parasite *Plasmodium* chabaudi, we identified a mutation in the mu chain of the AP2 adaptor protein complex (pcap2-μ) that arose along with the experimental evolution of artemisinin resistance. We screened several field isolates of *P. falciparum* from an ACT clinical trial in Burkina Faso, that were tested *in vitro* for their response to artemisinin derivatives and other drugs, and in pre- and post- treatment samples from an *in vivo* ACT trial carried out in Kenya, for genetic polymorphisms in the pfap2- μ orthologue. Genetic polymorphisms in pfap2- μ were analysed for association with several endpoints in both trials that might indicate a drug resistant parasite phenotype. Preliminary results indicate that polymorphisms in this adaptor protein subunit may be associated with *in vitro* and *in vivo* responses to artemisinin derivatives, quinine and lumefantrine. Further evaluation of pfap2-μ as a potential molecular marker of artemisinin resistance is now needed.

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DHFR AND DHPS SELECTIVE SWEEPS IN MALAWI AT A TIME OF HIGH SULFADOXIME-PYRIMETHAMINE USE

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Malawi and most other African nations have stopped using both chloroquine and sulfadoxine-pyrimethamine (SP) due to expansion of drug-resistant *Plasmodium falciparum* parasites. Directional selection of chloroquine resistance alleles in the form of a selective sweep has been shown, by analyzing variation in microsatellites flanking the chloroquine resistance gene, *pfcrt*. Similar selective sweeps of regions flanking *dhfr* and *dhps*, the genes that cause resistance to SP, have been identified in Africa, Southeast Asia, and South America. Here we report evidence of positive directional selection of *dhfr* and *dhps* resistance haplotypes and describe characteristics of the associated selective sweeps, at a time of high SP drug pressure in Malawi. Resistance alleles and flanking microsatellites were genotyped on 689 filter paper samples from children aged 6 months-12 years in Blantyre, Malawi from 1999-2001 when SP was the first-line treatment for malaria. All but one of the genotyped samples carried one or more SP resistance alleles. *Dhfr* triple-mutants conferring strong SP resistance predominated (51I/59R/108N), forming a quadruple mutant with *dhps* 540E. *Dhfr/dhps* quintuple mutants (*dhfr* 51I/59R/108N+*dhps* 437G/540E) were also observed. A reduction in microsatellite heterozygosity was identified in the regions flanking both *dhfr* and *dhps.* The sweep flanking *dhfr* extended from 10kb upstream to 20kb downstream of *dhfr*. The sweep flanking *dhps* extended from approximately 10kb upstream to at least 9kb downstream. Extended Haplotype Homozygosity was estimated, and showed increased linkage disequilibrium (LD) in regions flanking both genes relative to genomic levels of LD. Selective sweeps of resistant *dhfr and dhps* indicate that these alleles were under recent positive directional selection. The characteristics of the selective sweeps reported here, which were detected during a period of high SP drug pressure, will be compared to those detected after removal of SP as the first line therapy and in settings with different levels of malaria transmission.

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EVOLUTION OF DRUG RESISTANCE IN MALARIA PARASITES

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Efforts to relieve the burden caused by malaria rely critically on the availability of drugs targeting *Plasmodium falciparum*. The efficiency of these treatments is however seriously compromised by the appearance and spread of drug resistance. Resistance is observed today to some

extent against every available drug, including recent reports of resistance to artemisinin. Resistant strains of *Plasmodium falciparum* can spread in affected areas if they fare better than sensitive strains over the entire transmission cycle, including within-human and within-vector infection phases. Both hosts likely represent widely different environments for the parasite, particularly in terms of exposure to drugs and host-specific costs of resistance, which could notably affect the outcome of competition between resistant and sensitive strains and ultimately the evolution of drug resistance. To investigate this issue we present a model of malaria transmission combining between-hosts and within-hosts (human and vector) dynamics. The latter incorporates the impact of competition, treatment and immunity in a strain-specific fashion. We show how costs of resistance, particularly within-vector costs, affect the selection for resistant strains. We also explore how different drugs, acting on specific parts of the within-human cycle of *P. falciparum*, impact resistant strains (alone or in combination). Finally we also investigate the effect of vector control methods on the prevalence of resistance.

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IDENTIFYING DRUG RESISTANCE GENOTYPES IN ECUADORIAN MALARIA PARASITES

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Approximately 40% of the world population lives in malaria-endemic areas and recent estimates indicate that there are several hundred million cases and about 1.2 million deaths each year caused by this disease. Severe disease and resistance to antimalarials has been documented for *Plasmodium falciparum* and *P. vivax* and efforts to control malaria have become more challenging in recent years due to widespread drug resistance. Today, the vast majority of *P. falciparum* isolates in Latin America are resistant to chloroquine (CQ) and other drugs and resistance to CQ has been reported for *P. vivax*. It is also widely recognized that drug resistance has played a role in the reemergence of malaria in the Amazon basin at the end of the 20th century. The antimalarial resistance situation in Ecuador is not well known and genotypes for drug resistance from different parts of the country have not been studied. In order to identify and analyze genotypic markers for drug resistance in Ecuador we are doing PCR-RFLP from confirmed malaria blood samples spotted in filter paper using specific primers for *Pfcrt*, *Pfdhfr* and *Pfdhps* (*P. falciparum*) and *Pvdhfr*, *Pvdhps* (*P. vivax*). Our results so far show that the tested Ecuadorian *P. falciparum* isolates have a mutant *PfCRT*. In addition, we will present *P. falciparum* and *P. vivax* genotype data from different resistance markers. The study of the prevalence of drug resistance in Ecuadorian *P. falciparum* and *P. vivax* will enhance our knowledge of drug resistance in Latin America, a necessary task to improve the way malaria is treated in this region of the world.

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PERSISTENT *PLASMODIUM FALCIPARUM* INFECTIONS DRIVE EXPANSION OF ATYPICAL MEMORY B CELLS AS WELL AS EXHAUSTED T CELLS

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Recent epidemiological and animal studies suggest that *Plasmodium* parasites induce atypical/exhausted lymphocytes in their hosts, perhaps as an immune evasive strategy. Whilst these immunoregulatory lymphocytes may benefit the host by checking exaggerated immune responses and hence reducing immunopathology, they may also impede generation of protective immune responses. Thus such observations may explain in part: why naturally acquired immunity to malaria develops slowly, often requiring several years of repeated exposure to become effective, and why vaccines confirmed protective in animals and naïve volunteers fail to protect malaria-exposed individuals. Here, we compared frequencies of atypical memory B cells (MBC) and exhausted T cell phenotypes between well-characterised cohorts of children of similar genetic backgrounds and living in similar environmental conditions, but whose rate and/history malaria exposure differs. We confirm that current malaria exposure drives expansion of atypical MBCs, and provide evidence suggesting that these Pf-associated atypical MBCs are expanded at the expense of naïve B cells. We show that persistent Pf exposure drives expansion of both PD-1 single, and PD-1 and Lag-3 double positive exhausted CD4 T cells, and to a lesser extent single-positive LAG-3 positive exhausted CD4 T cells. This expansion of PD-1, and double PD-1 and LAG-3 positive CD4 T cells is largely confined to CD45RA positive cells. The percentage of PD-1 and Lag-3 double positive CD45RA positive CD4 T cells correlated negatively with frequencies of activated and classical MBCs. Single PD-1, and double PD-1 and LAG-3 positive CD8 T cells were increased among the total, and TEFF CD8 T cells, respectively, but only in the presence of asymptomatic parasitaemia. Together, these results suggest that Pf drives expansion of atypical lymphocytes. The implication is that these cells may dampen inflammatory responses to malaria, thus reducing pathogenesis, but may also impede the generation of protective responses.

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THE EFFECT OF MATERNAL MALARIA AND HELMINTH INFECTIONS ON CHILDHOOD MALARIA: A BIRTH COHORT IN ENTEBBE, UGANDA

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Helminths and *Plasmodium* infections are common in the tropics, and positive associations have been observed between these parasitic infections in pregnancy. While malaria in pregnancy has been associated with adverse maternal and birth outcomes, knowledge on the effect of prenatal exposure to malaria and helminth infections on childhood malaria is still sparse. This study took place in Entebbe, Uganda. 2507 women were recruited in a trial on the effects of albendazole and praziquantel in pregnancy. Blood and stool samples were examined for helminth and *P. falciparum* infections. The offspring were followed up to age five years, and their malaria morbidity data collected prospectively. Clinical malaria was diagnosed as fever (≥ 37.5^{0}C) with *P. falciparum* parasitaemia, and asymptomatic parasitaemia recorded annually at scheduled visits. In multivariate analyses we adjusted for risk factors associated with malaria and helminth infections. Common parasitic infections in pregnancy were hookworm (45%), *Mansonella perstans* (21%), *Schistosoma mansoni* (18%), and *P. falciparum* (11%). Of 2345 liveborn infants, 69% were still under follow-up at age 5 years. The overall childhood malaria rate was 34 episodes per 100 child-years, and the cumulative prevalence of asymptomatic *P. falciparum* parasitaemia over the five years was 5%. Maternal hookworm and *M. perstans* infections were associated with an increased risk of childhood malaria (adjusted Hazard Ratio [aHR] 1.26, p<0.001 and 1.23, p=0.004 respectively), and increased cumulative prevalence of asymptomatic parasitaemia (adjusted Odds Ratio [aOR] 1.59, p=0.001 and 1.55, p=0.01 respectively). *S. mansoni* infection showed no such associations. Maternal *P. falciparum* infection was associated with an increased risk of childhood malaria (aHR 1.22, p=0.04) but not prevalence

of asymptomatic malaria (aOR 1.21, p=0.4). This study shows that the effect of malaria in pregnancy on childhood malaria extends to age five years, and is the first report of an association between helminth infections in pregnancy and malaria in the offspring.

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VALIDITY OF SELF-REPORTED USE OF SULFADOXINE-PYRIMETHAMINE INTERMITTENT PRESUMPTIVE TREATMENT DURING PREGNANCY (IPTP): A CROSS-SECTIONAL STUDY

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Malaria in pregnancy is a major health problem that can cause maternal anaemia, stillbirth, spontaneous abortion, low birth-weight and intra-uterine stunting. The WHO recommends use of Sulphadoxine-Pyrimethamine (SP) for Intermittent Preventive Treatment of malaria during pregnancy (IPTp) in endemic areas. Towards monitoring and assessing IPTp coverage in the population, the Roll Back Malaria program recommends use of self reported data. In this study, we assessed the validity of self reported use of IPTp by testing for sulfadoxine in maternal blood at delivery. Two hundred and four pregnant women were consented and enrolled in a cross-sectional study. We excluded participants who reported a history of taking sulfa containing drugs, those who were not sure of dates relating to last menstrual period or who took IPTp before 20 weeks of gestation. Data on demographic characteristics, obstetric history, and delivery outcome were collected. At delivery of the baby, we took the mother's venous blood, carried out blood smear microscopy for parasites and tested the plasma for sulfadoxine using High Performance Liquid Chromatography (HPLC). We found that 17.2% of participants reported to have used IPTp and indeed tested positive by HPLC while 30.4% reported not to have used IPTp and indeed tested negative for sulphadoxine. Participants possessing post primary education were more likely to have reported using IPTp. The low agreement between self report and actual presence of the drug in the blood casts doubt on the validity of self reported data in estimating IPTp coverage. We recommend further research of self reported data towards improving the accuracy of such information which is vital for guiding policy for malaria control in pregnancy since routine blood drug assays would be too expensive and impractical for population based studies.

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A SPATIO-TEMPORAL ANALYSIS OF *PLASMODIUM FALCIPARUM* AND *P. VIVAX* INFECTIONS AND TREATMENT SEEKING BEHAVIOR IN THA SONG YANG DISTRICT, TAK PROVINCE, THAILAND: 2008-2011

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Despite being relatively successful at controlling malaria in most of the country, the border areas surrounding Thailand continue to experience persistent, seasonal malaria. The heaviest malaria burden within Thailand is along the Thai-Myanmar border in Tha Song Yang District, Tak Province. This area is also a center of drug and multi-drug resistant malaria and recently decreased parasite sensitivity to artemisinin derivatives (largely considered the last defense against drug-resistant malaria) has been reported from Tak Province. Here we use exploratory spatial analysis and accelerated failure time models to evaluate spatio-temporal patterns in both *Plasmodium falciparum* and *P. vivax* case frequency and treatment seeking behavior. We are specifically interested in potential clustering of cases near the Thai-Myanmar border as well as the length of time between a patient's reported onset of malaria symptoms and the time they actually visit a malaria clinic. Our temporal analysis is at the subdistrict level (within Tha Song Yang District) whereas our spatial analysis is at both the subdistrict and district levels (within Tak Province.) We find a general pattern of spatial decay, with general correspondence in both parasite species, and with the heaviest case-loads clustered in administrative units that touch the Thai-Myanmar border. However, this pattern isn't a smooth gradient from the border towards central Thailand. Finally, our temporal analyses indicate an initial clustering of treatment seeking times around 2 to 3 days and several other clusters occurring after 8 days. For example, among those that seek treatment within 7 days after the onset of symptoms, Myanmar nationals are the quickest to seek treatment. Conversely, among those that wait until after a week of experiencing symptoms Myanmar nationals wait the longest to seek treatment. This discordance in treatment seeking behavior has important implications for public health and global health. Individuals who are carrying parasites in their blood for longer periods of time may increase the risk of infection for the populations surrounding them. These results are significant with regards to the increased potential of transmitting drug (potentially artemisinin) resistant malaria.

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UNDERSTANDING THE IMPACT OF SUBSIDIZING ARTEMISININ-BASED COMBINATION THERAPIES (ACTS) IN THE RETAIL SECTOR - RESULTS FROM FOCUS GROUP DISCUSSIONS IN RURAL KENYA

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There is considerable interest in the potential of private sector subsidies to increase availability and affordability of artemisinin-based combination therapies (ACTs) for malaria treatment. A cluster randomized trial of such subsidies was conducted in 3 districts in Kenya, comprising provision of subsidized packs of paediatric ACT to retail outlets, training of retail staff, and community awareness activities. The results demonstrated a substantial increase in ACT availability and coverage, though patient counselling and adherence were suboptimal. We conducted a qualitative study in order to understand why these successes and limitations occurred. Eighteen focus group discussions were conducted, 9 with retailers and 9 with caregivers, to document experiences with the intervention. Respondents were positive about intervention components, praising the focused retailer training, affordable pricing, strong promotional activities, dispensing job aids, and consumer friendly packaging, which are likely to have contributed to the positive access and coverage outcomes observed. However, many retailers still did not stock ACT, due to insufficient supplies, lack of capital and staff turnover. Advice to caregivers was poor due to insufficient time, and poor recall of instructions. Adherence by caregivers to dosing guidelines was sub-optimal, because of a wish to save tablets for other episodes, doses being required at night, stopping treatment when the child felt better, and the number and bitter taste of the tablets. Caregivers used a number of strategies to obtain paediatric ACT for older age groups. In conclusion, this study has highlighted that important components of a successful ACT subsidy intervention are regular retailer training, affordable pricing, a reliable supply chain and community mobilization emphasizing patient adherence and when to seek further care.

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COMPARISON OF MALARIA RISK FACTORS AND PARASITEMIA AMONG CHILDREN LIVING EITHER WITH NON-PARENT GUARDIANS OR WITH BIOLOGICAL PARENTS: ANALYSIS OF 2009 UGANDA MALARIA INDICATOR SURVEY DATA

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As of 2009, approximately 2.7 million child orphans were living with one parent or non-parent guardians (NPG) in Uganda. These children may be at a higher risk of malaria than children living with their biological parents (BP) due to possible differences in access to malaria prevention measures and household characteristics. The 2009 Uganda Malaria Indicator Survey collected malaria prevention coverage and household data from 4,760 nationally representative households, and blood smear samples for malaria parasitemia from children under 5 years old (U5) living in those households. Data were analyzed in SAS 9.2 (proc surveylogistic, surveyfreq). Odds ratios (OR) of main outcome measures and associated 95% confidence interval (CI) and p-values (p) were computed. Children under 5 years old were categorized as either living with NPG or BP based on their relationship to the head of the household. During the 2009 MIS, 707 (18%) out of 3933 U5 were living with NPG. NPG head of the household were likely to be older [median age; 54 years, IQR: 28-40 vs. 34, IQR: 47-63; p<.01] and female [54% vs. 18%, p<.01]. Fewer NPG households owned at least one insecticide treated net [76%, 95% CI: 71-81 vs. 80%, 95% CI: 77-84; p=.33]; and fewer NPG children slept under any bednet the night before the survey (bednet use) [58%, 95% CI: 50-67 vs. 75%, 95% CI: 72-79; p<.01]. Adjusting for children's age and head of household's age, NPG children were less likely to use any bednet the night before the survey than BP children (OR: 0.6, 95% CI: 0.4-0.9; p<.02). Adjusting for children's age, head of household's age and sex, household wealth quintile, and bednet use, the odds of malaria parasitemia was four times greater for NPG children than BP children (OR: 4.2, 95% CI: 1.8-9.7; p<.01). The odds of parasitemia among NPG children were strongly modified by the interaction with age of head of household and bednet use (p for interactions<.001). NPG children may be at a greater risk for malaria than BP children, and may warrant special targeting of malaria intervention efforts.

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LOW PREVALENCE OF PLACENTAL MALARIA INFECTION AMONG PREGNANT WOMEN IN ZANZIBAR: POLICY IMPLICATIONS FOR IPTP

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Efforts by the Zanzibar Ministry of Health to scale-up malaria prevention and treatment strategies, including intermittent preventive treatment for pregnant women (IPTp), have brought Zanzibar to the pre-elimination phase of malaria control. *P. falciparum* prevalence in the general population has been below 1% since 2008 and the diagnostic positivity rate among febrile patients was 1.2% in 2011. Zanzibar implemented IPTp using sulfadoxine-pyrimethamine (SP) in 2004 when malaria prevalence exceeded 20%. While coverage among pregnant women is low (47% received two doses SP), the value of this intervention in low transmission settings remains uncertain. Few countries in Africa have confronted policy questions regarding timing of IPTp scale-down. We designed a prospective observational study to estimate prevalence of placental malaria among pregnant women with no evidence of receiving any dose of SP for IPTp during pregnancy. From September 2011 to April 2012 we enrolled a convenience sample of pregnant women on day of delivery at six hospitals in Zanzibar (three in both Pemba and Unguja). Dried blood spots (DBS) on filter paper were prepared from placental blood specimens. DBS were analyzed via polymerase chain reaction indicating active *Plasmodium* infection (all species). To date, over 1,200 deliveries were enrolled at the six recruitment sites (approximately 12% of total, range: 8-26%). Two (0.19%; 95% CI, 0.05-0.69%) of 1,046 DBS specimens analyzed to date showed evidence of *P. falciparum* infection. Both were from HIV uninfected, multigravid women in Unguja. Birth weights for both deliveries were normal (>2500 g). Data collection will continue through the peak transmission season of May-July 2012. The very low prevalence of placental infection among women who received no IPTp raises policy questions regarding continuation of IPTp in Zanzibar. Alternative efforts to control malaria in pregnancy in Zanzibar, such as active case detection via regular screening and treatment during antenatal visits, should be evaluated.

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FINE-SCALE SPATIAL VARIATION IN TRANSMISSION INTENSITY, IN SECULAR TRENDS OF TRANSMISSION INTENSITY, AND IN THE AGE PROFILE OF FEBRILE MALARIA IN KILIFI, KENYA

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Malaria transmission is spatially heterogeneous. Maps of malaria episodes at fine spatial scales often show clusters of transmission comprising groups of homesteads or "hotspots". These hotspots make malaria control measures less effective than might have been expected, but targeting intensive control interventions at the hotspots could be highly effective. At present, there are few epidemiological descriptions of the properties of hotspots. We have previously shown that hotspots of asymptomatic parasitaemia are stable over several years, but hotspots of febrile malaria are unstable. The risks of asymptomatic parasitaemia and febrile malaria were closely related to proximity of *Anopheles* larval sites, interacting with wind direction. We hypothesise that immunity offsets the high rate of febrile malaria that might otherwise occur in stable hotspots, whereas unstable hotspots necessarily affect a population with less prior exposure to malaria. We present data from 4,200 episodes of malaria among 4,800 homesteads monitored from a local dispensary in Kilifi, Kenya, from 2003 to 2011. There was marked spatial clustering of febrile malaria episodes. Spatial clustering of febrile malaria among younger children was more stable over time compared with among older children. Reasoning that febrile malaria risk in younger children was less confounded by immunity, we used data from children below one year of age to classify homesteads into high or low mean transmission intensity, and into rising or falling secular trends of transmission intensity. This classification predicted the age-profiles of febrile malaria by homestead. At high mean transmission, the peak febrile malaria risk was at 3 years of age, and at low mean transmission intensity the peak febrile malaria risk was at 8 years of age. A rising secular trend of transmission predicted a sustained risk of malaria in children above 10 years of age, whereas a falling secular trend predicted a falling risk of malaria. We conclude that aggregated febrile malaria incidence is inadequate to represent the complexity of

spatial heterogeneity of transmission intensity and secular trends in transmission intensity. Our results indicate fine scale spatial variation in the epidemiology of malaria in a 150 sq km area that was comparable with the variation seen on more coarse spatial scales across regions or countries.

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ESTIMATING TRANSMISSION INTENSITY FROM *PLASMODIUM FALCIPARUM* SEROLOGICAL DATA USING ANTIBODY DENSITY MODELS

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Serological data are increasingly being used to monitor malaria transmission intensity and have been demonstrated to be particularly useful in areas of low transmission where traditional measures such as EIR and parasite prevalence are limited. The seroconversion rate is usually estimated using catalytic models in which the measured antibody levels are used to categorise individuals as seropositive or seronegative. One limitation of this approach is that the cut-off between positive and negative is arbitrary. Furthermore, the continuous variation in antibody levels is ignored thereby potentially reducing the precision of the estimate. To overcome these limitations we developed a series of age-specific density models which mimic antibody acquisition and loss. These were fitted to antibody titre data from multiple *Plasmodium falciparum* endemic settings to estimate the rate of acquisition of antibodies as an alternative measure of transmission intensity. Our results indicate that a model in which the boost in antibodies following exposure depends on the existing titre (with an exponential decline in the size of the antibody boost with higher levels of circulating antibodies) and that includes variation between individuals in the size of the response fits the data well. Furthermore our results show a consistent ordering of transmission intensities compared to those from a catalytic model. This approach, if validated across different epidemiological settings, could be a useful alternative model for measuring transmission intensity which avoids the need for an arbitrary cut-off value.

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PREVALENCE OF MALARIA AND ANEMIA AMONG PREGNANT WOMEN ATTENDING ANTENATAL CARE CLINICS IN THE EJISU-JUABEN AND SEKYERE-EAST DISTRICTS OF GHANA

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Malaria and anaemia (haemoglobin concentration < 11g/dl) in pregnancy continue to be of public health importance in Ghana with malaria contributing to 9.4% of maternal deaths. Strategies are being implemented through the antenatal care system to reduce their occurrence but asymptomatic malaria parasitaemia and anaemia prevalence at term stand at 12.1% and 45.0% respectively. In preparation for a cluster randomised control trial to determine the effect of an enhanced antenatal care package on malaria and anaemia in pregnancy, a cross-sectional study was conducted from December 2011 to April 2012 among pregnant women with gestation ≥32 weeks and prior to delivery. Trained research assistants determined malaria parasitaemia and haemoglobin concentration levels using the malaria rapid diagnostic test and the HemoCue 301. An interviewer guided questionnaire was also administered to determine the demography, bed net use, IPTp administration and self-reported adherence to iron and folate supplementation among the pregnant women. The prevalence of malaria parasitaemia and anaemia was 15.5% and 42.6% respectively. Parasitaemia occurred in a significantly younger age group (25.1 (6.21) yrs vs 27.4 (6.29) yrs; p=0.007) and these had a significantly lower haemoglobin concentration (10.5 (1.37) g/dl vs 11.2 (1.29) g/dl; p<0.0001). Although 61.2%of the pregnant women owned bed nets, only 39.3% slept under one during the night before the survey. A total of 81.2% received two or more doses of SP and 50.5% reported high adherence to iron and folate supplementation however these were not significantly associated with the prevalence of parasitaemia or anaemia. Malaria parasitaemia and anaemia are still prevalent in the study area despite the implementation of current strategies including ITN use, SP-IPTp, iron and folate supplementation and prompt diagnosis and effective treatment of malaria. Probably new ways of delivering these strategies to make them more effective need to be explored.

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REVITALIZING ROUTINE HEALTH FACILITY DATA FOR MALARIA CONTROL

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Successful scale up of proven malaria control interventions across sub-Saharan Africa since 2000 has resulted in rapid changes in malaria epidemiology. Because of these changes, national malaria control programs and their partners need effective tools to adequately monitor malaria burden for surveillance and program planning. Existing tools, most notably national-level household surveys such as the Malaria Indicator Survey and Demographic and Health Survey, do not provide information on longitudinal changes in malaria burden. Cross-sectional data do not reflect seasonal fluctuations in malaria burden nor typically provide district- or sub-district-level data useful for program monitoring and planning. In many countries, routine health facility data are of unknown validity due to reporting of clinical diagnosis without laboratory confirmation and incomplete or late reporting. Recognizing the need for improved approaches to measure longitudinal changes in malaria burden, the authors have undertaken a comprehensive examination of routine health facility data and are optimistic about its potential to complement existing data for malaria control. Using models from Benin, Ethiopia, Madagascar, and Uganda, the authors have critically assessed the strengths and weaknesses of different health facility-based surveillance systems and created a framework to assist countries in developing robust data collection systems that will meet country-specific data needs while taking into account resource limitations. The framework guides stakeholders in the decision-making process and is comprehensive in that it considers scope, indicators, data collection tools, supervision, and data use. The anticipated outcomes of providing a framework for strengthening facility-based data collection systems for malaria include increasing the quality of routine system data, improving country capacity for planning, and sustaining the progress made in malaria control over the past decade.

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MOLECULAR EPIDEMIOLOGY OF *PLASMODIUM VIVAX* RELAPSES IN THE PERUVIAN AMAZON

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Plasmodium vivax accounts for 71-81% of malaria cases in the Americas. To determine the magnitude of *P. vivax* relapsing malaria in rural Amazonia, we carried out a study from 2005-2008 in four health posts in the Amazonian Region of Loreto in northeast Peru where the majority of nationally reported malaria cases originate. PCR-restriction fragment length polymorphism of *PvMSP-3α* (enzymes Hha1 and Alu1) and PCR of nine tandem repeat markers were compared for their ability to distinguish relapse vs. reinfection. Of 1507 subjects with *P. vivax* malaria, 354

developed >1 episode during the study. 97/354 (27.4%) were defined as relapses using *Pvmsp-3α* alone. Adding tandem repeat polymorphism analysis significantly reduced the number of definitively-defined relapses to 26/354 (7.4%) ($p<0.05$), allowing for more new infections to be identified. Odds of another episode of *P. vivax* malaria, whether due to relapse or reinfection, were 2.6 times higher in the more remote village of Mazan than in villages closer to Iquitos city ($p<0.001$) (OR=2.6, 95%CI: 2.0,3.4). People in Mazan were 2.4 times more likely to develop a relapse (not reinfection) than people in other villages (OR=2.4, CI95%:1.1,5.5; p=0.03). The proportion of multiple genotype infections was 16.1% by TR, 4.5% by MSP-3α, and 18.8% using both. The use of highly resolving molecular markers of *P. vivax* allowed for finding an unexpectedly high proportion of multiple genotype infections, remarkable considering the current knowledge of transmission intensity and entomological inoculation rates in the region. Highly discriminatory molecular epidemiological tools will allow us to gain critical knowledge of the micro-geography of malaria transmission in this area of low transmission

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TEMPORAL TRENDS IN SEVERE MALARIA IN CHITTAGONG, BANGLADESH

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Epidemiological data on malaria in Bangladesh are sparse, particularly on severe and fatal malaria. This hampers the allocation of healthcare provision in this resource-poor setting. Over 85% of the estimated 150,000-250,000 annual malaria cases in Bangladesh occur in Chittagong Division with 80% in the Chittagong Hill Tracts (CHT). Chittagong Medical College Hospital (CMCH) is the major tertiary referral hospital for severe malaria in Chittagong Division. Malaria screening data from 22,785 inpatients in CMCH from 1999-2011 were analysed to investigate the patterns of referral, temporal trends and geographical distribution of severe malaria in Chittagong Division. From 1999 till 2011, 2,394 malaria cases were admitted, of which 96% harboured *Plasmodium falciparum* (Pf) and 4% *P. vivax* (Pv). Infection was commonest in males (67%) between 15 and 34 years of age. Seasonality of malaria incidence was marked with a single peak in *P. falciparum* transmission from June to August coinciding with peak rainfall, whereas *P. vivax* showed an additional peak in February-March likely representing relapse infections. Since 2007 there has been a substantial decrease in the absolute number of admitted malaria cases. Case fatality in severe malaria was 18% from 2008-2011 remaining steady during this period. A travel history obtained in 220 malaria patients revealed only 34% had been to the CHT in the preceding 3 weeks. Of all admitted malaria patients, only 9% lived in the CHT, but none in the more remote malaria endemic regions near the Indian border. The overall decline in admitted malaria cases to CMCH suggests recent control measures are successful. However, there are no reliable data on the incidence of severe malaria in the CHT, the most endemic area of Bangladesh, and most of these patients do not reach tertiary health facilities. Improvement of early treatment and simple supportive care for severe malaria in remote areas and implementation of a referral system for cases requiring additional supportive care could be an important component of further reducing malaria-attributable disease and death in Bangladesh.

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SOCIO-DEMOGRAPHICS AND THE DEVELOPMENT OF MALARIA ELIMINATION STRATEGIES IN THE LOW TRANSMISSION SETTING

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This analysis presents a comprehensive description of malaria burden and risk factors in Peruvian Amazon villages where malaria transmission is hypoendemic. More than 9000 subjects were studied in contrasting village settings within the Department of Loreto, Peru, where most malaria occurs in the country. *Plasmodium vivax* is responsible for more than 75% of malaria cases; severe disease from any form of malaria is uncommon and death rare. The association between lifetime malaria episodes and individual and household covariates was studied using polychotomous logistic regression analysis, assessing effects on odds of some vs. no lifetime malaria episodes. Malaria morbidity during lifetime was strongly associated with age, logging, farming, travel history, and living with a logger or agriculturist. Select groups of adults, particularly loggers and agriculturists acquire multiple malaria infections in transmission settings outside of the main domicile, and may be mobile human reservoirs by which malaria parasites move within and between micro-regions within malaria endemic settings. For example, such individuals might well be reservoirs of transmission by introducing or reintroducing malaria into their home villages and their own households, depending on vector ecology and the local village setting. Therefore, socio-demographic studies can identify people with the epidemiological characteristic of transmission risk, and these individuals would be prime targets against which to deploy transmission blocking strategies along with insecticide treated bednets and chemoprophylaxis.

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FINE-SCALE SPATIAL HETEROGENEITY OF DRY SEASON PREVALENCE AND ENVIRONMENTAL RISK OF MALARIA AMONG CHILDREN IN RURAL MALAWI: A CASE-CONTROL STUDY

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Environmental risk factors for malaria during the dry season in rural Malawi remain relatively unstudied. We investigated the role of local environmental features, in particular active smallholder agricultural land, on malaria prevalence among children <5 years old living in villages in two neighboring rural Traditional Authority (TA) regions in southern Malawi during the dry season. Ten villages from TA Sitola and Msamala were randomly selected. All houses with children <5 were approached and informed consent was obtained from those who agreed to participate, after which the house location was recorded with GPS. At each participating house, a nurse administered a malaria rapid diagnostic test (RDT) to one child, and a questionnaire to parents. Environmental data were collected in and around each house, including land cover <50 meters. Environmental variables found to be significantly associated with RDT status (+/-) at $p<0.10$ in bivariate analysis (X^2 or Student's t-test) were analyzed with multi-level multivariate logistic regression (MLLR). Geographic clustering of RDT status, environmental factors, and Pearson residuals from MLLR were calculated using the Getis-Ord Gi* statistic. A total of 390 children were enrolled from six villages in Sitola (n=162

households) and four villages in Msamala (n=228), of whom 178 (45.6%) tested malaria positive. MLLR was used to evaluate associations of RDT status and household proximity to agriculture (<25m radius), controlling for child sex and age (months), bed net ownership, elevation (meters), and random effects intercepts for village and TA-level unmeasured factors. Proximity to active agriculture was a significant predictor of being malaria positive (OR 2.80, 95% CI 1.41-5.55). Mapping of Pearson residuals from MLLR showed significant clustering (Gi* z>2.58, p<0.01) predominantly within TA Sitola, with a somewhat different pattern in TA Msamala on the other side of the Shire River. Evidence shows significant spatial heterogeneity of malaria prevalence and risk factors at very fine scales in this rural Malawi setting, suggesting the need to focus intervention efforts.

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GENOTYPIC PATTERNS OF RELAPSING *PLASMODIUM VIVAX* INFECTIONS IN CAMBODIA

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The propensity for *Plasmodium vivax* parasites to relapse is one of the major obstacles to malaria control and elimination in many regions of the world. Yet little is known about the nature of relapse. A key unanswered question is whether certain *vivax* variants are more likely to cause relapse, as many infections contain multiple variants. Using a newly developed *P. vivax* heteroduplex tracking assay (HTA) targeting *P. vivax* merozoite surface protein 1 (*Pvmsp*1), we genotyped 107 *vivax* infections in individuals from Chumkiri, Cambodia, 45 of whom developed recurrent parasitemia between day 28 and day 42 following chloroquine treatment without primaquine. The HTA, which is adept at uncovering minority variants, revealed multiple coinfecting genotypes in 83% of individuals, with a mean multiplicity of infection (MOI) of 2.8 (IQR 2-4). Genotypes of paired initial and recurrent parasitemias were compared to look for genotypic patterns of relapse. Despite high allelic diversity in the overall cohort (H_E = 0.86), 86% (38/44) of paired isolates were highly related, sharing at least half their variants. At the same time, novel variants appeared in 30% (13/44) of recurrent isolates. When the genotypes from initial infections of 45 "relapsers" and 62 "nonrelapsers" (those who did and did not develop recurrent parasitemia within 42 days) were compared, two specific *Pvmsp1* variants were associated with subsequent relapse. By accounting for the polyclonality of *P. vivax* in Cambodia, we find a complex scheme of relapse in which hypnozoites representing all or a subset of the multiple clones found in an initial infection can reactivate in concert to cause relapse. At the same time, the common appearance of novel variants supports the notion that latent hypnozoites may be reactivated at the time of relapse. Additionally, we have identified individual *Pvmsp1* variants that demonstrate a greater propensity for early relapse, suggesting a genetic basis to relapse.

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REPRESENTATIVENESS, COMPLETENESS, TIMELINESS AND ACCURACY OF ZANZIBAR'S MALARIA EPIDEMIC EARLY DETECTION SYSTEM (MEEDS), 2008-2011

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Zanzibar's recent population-based survey estimates of malaria prevalence (<1%) and diagnostic test positivity rates of parasitemia among febrile outpatients (<2%) are approaching pre-elimination levels. In 2008 Zanzibar developed and implemented a mobile phone-based malaria epidemic early detection system (MEEDS) at peripheral clinics to facilitate weekly reporting of confirmed malaria cases and help ensure prompt detection, confirmation, and response to sudden increases in *Plasmodium falciparum* transmission. Our objective was to analyze 2008-2011 MEEDS data and describe trends in several MEEDS attributes related to outbreak detection. System representativeness was the proportion of all public clinics in Zanzibar reporting data to MEEDS in a given year. Completeness of reporting was defined as submission of all weekly data elements submitted to the system, regardless of date. Timeliness of reporting was calculated as the proportion of expected reports received by the system by Monday of the following week. Finally, data accuracy was assessed through a manual count of weekly case totals from the routine health management information system (HMIS) registers compared to totals in MEEDS registers. Representativeness improved as MEEDS implementation moved forward from 10 (7%) clinics in 2008 to 52 (37%) in 2009, 69 (49%) in 2010, 90 (63%) in late 2010, and finally 142 (100%) clinics by late 2011. Completeness of submitted data was 100% each year except 2009 (84%) when technical problems prevented data transmission from many clinics. Timeliness of weekly reports received by the following Monday increased from 19% in 2009 to 43% in 2011 (p<0.001). The MEEDS data accuracy as compared to routine HMIS increased from 89% in 2009 to 97% in the first-half of 2011 and fell to 93% in the second-half of 2011. Despite accomplishments in reporting representativeness, completeness, and accuracy of the MEEDS over four years of implementation, additional efforts and resources are required to understand and address deficiencies in reporting timeliness, perhaps the most important attribute of an early epidemic detection system.

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DECREASE OF MALARIA INCIDENCE AMONG CONFIRMED CASES OF MALARIA IN MARY IMMACULATA CENTRE MUKURU KENYA IN 2007-2010

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The aim of the study was to assess annual incidence of microscopically positive cases of malaria in urban area of Nairobi within last five years (2007-2011) and to compare malaria occurrence before and after intermittent preventive treatment (IPT) was initiated in this area. Mary Immaculata Centre is located in the slum area of Mukuru (Nairobi, Kenya) with about 40 000 inhabitants in height of 1900 meters above sea level. Traveling from Nairobi to down country of Rift Valley and back is usual 1-2 times per year. Two experienced lab technicians investigated daily 30-50 slides a day (15-25 per person). In 2011 also rapid diagnostic tests

(RTD) were used to confirm positive malaria slide. Within five years (2007-2011), 56 668 samples were microscopically evaluated (8466 - 12333 per year) and 905 were positive for malaria (1,60%). Annual proportion decreased from 1,96% (2007), 2,54% (2008) and 2,11% (2009) to 1% in 2010 and 0,79% in 2011 (P<0,001). Severe cases of malaria were seen only exceptionally. Number of cerebral malaria cases was 1-5 patients/year and severe anaemia (<80 g/l) was also exceptional (15-30 cases/year). Decreasing proportion of microscopically positive malaria cases was probably due to major improvement in infrastructure (disinfection of surface water, canalization, waste water drainage) as well as due to IPT in all four schools in Mukuru since 2009/2010 in all children coming to first school year age and also for all mothers coming to maternity check since 2009. Seasonal variation has been observed as well with maximum in June - October (rainy season) and minimum in November - December. In conclusion, decrease of annual incidence of microscopically positive cases of malaria in 2010-2011 has been observed in slum area of Mukuru in Nairobi, Kenya. Sewage water drains and canalizations of surface water in this area as well as IPT in school children and pregnant women may play a role in this trend within last five years.

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ESTIMATES OF MALARIA MORBIDITY BEFORE AND AFTER THE IMPLEMENTATION OF A SENTINEL SITE INPATIENT MALARIA SURVEILLANCE SYSTEM IN UGANDA

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In Uganda, the National Malaria Control Program (NMCP) relies on Health Management Information System (HMIS) data for planning and monitoring trends in malaria burden yet majority of malaria cases reported are based on clinical case definition. For the past 2 years, we have implemented an in-patient malaria sentinel surveillance program at select district hospitals with emphasis on laboratory-based case definition. To better characterize the quality of HMIS-based malaria data, and understand the true burden of malaria in Uganda, we compare HMIS data to malaria sentinel surveillance site data at four public hospitals. These hospitals are situated in districts with varying malaria endemicity: Tororo and Apac (high transmission), Mubende (medium transmission) and Kambuga (low transmission). At the four sentinel hospitals, >95% of inpatient children less than 5 years were tested for malaria, and only those children with positive laboratory confirmation were recorded as malaria cases. Based on HMIS data, the proportion of hospitalized children under 5 with malaria was higher 12 months prior to start of the program as compared to 12 months after: Tororo (94% vs. 85%), Kambuga (83% vs. 52%), Mubende (71% vs. 55%) and Apac (67% vs. 40%). Actual comparison of HMIS data to surveillance program data, 12 month after its start, showed that HMIS data overestimates the burden of malaria when compared to surveillance program data: 27 percent higher in Kambuga (25% vs. 52%), 24 percent higher in Tororo, (61% vs. 85%), 18 percent higher in Mubende (55% vs. 37%) and 7 percentage points higher in Apac (42% vs. 35%). Improved precision of HMIS estimates of malaria adopted after start of the program may have contributed, in addition to other factors, to the observed differences in disease burden determined by HMIS before and after the start of the program. Even then, HMIS overestimated the burden of malaria among hospitalized children after start of the program. In order to improve the quality of HMIS malaria data, a case definition based on laboratory confirmation should be adopted.

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MALARIA AND RESPIRATORY TRACT INFECTIONS WERE THE COMMONEST TROPICAL DISEASES AND THE COMMONEST INFECTIONS IN AREA OF LOW HIV PREVALENCE IN SOUTH UGANDA: ANALYSIS OF 43,551 PATIENTS

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The aim of this study was assess reasons for hospitalization in rural hospital in South Uganda in area of low HIV prevalence among population (2-3 % HIV positivity). Since January 2008 to December 2010 all 43 550 patients has been investigated, of them 6454 (14,8 %) inpatients and 37 096 (85,2 %) outpatients. Total 32 938 (75,6 %) were children under 5 years of age (2965 inpatients). Rapid diagnostic test plus microscopy has been used in the hospital laboratory with four florescent microscopes and six experienced laboratory technicians (analysis about 100-120 test/samples per day). Total 20 421 (46,9 %) of malaria cases within 3 years (2008-2010) were diagnosed. Of all malaria patients, 17 321 (84,8 %) were treated on outpatients and 3100 (15,2 %) on inpatients basis. Altogether 31 960 blood smears were microscopically investigated and 16 205 (50,7 %) of those were positive. Respiratory tract infections were diagnosed in 9255 cases (21,3 %), of them 3422 (36,9 %) had pneumonia and 5833 (63,1%) lower respiratory tract infections. Otitis media (862 cases) was observed only in children. Tuberculosis was confirmed in 102 patients and 30 of them were HIV co-infected. Other frequently diagnosed infections were skin and soft tissue infections in 3383 patients (7,8 %), urogenital tract infections in 3145 (7,2%), sexually transmitted infections 966 (2,2 %), of them 241 (24,9%) laboratory confirmed cases of syphilis. Totally 2126 patients (4,9%) have microscopically diagnosed geohelmints infections and 1387 (3,2%) had diarrhea. Together 4513 patients were tested on HIV and 1188 (2,7 %) of these were positive. Another diseases with low prevalence were ocular infections in 793 (1,8 %) patients, meningitis in 53 patients (0,1 %), measles in 14 (0,03%), schistosomiasis in 12 (0,03 %), sleeping sickness in 10 patients (0,02 %) and tetanus in 12 (0,03%) patients (4 of them neonates). In the area of Buikwe (Lugazi, Buikwe District, South Uganda), prevalence of HIV was surprisingly low (2,7%) as well as geohelmints infections (3,2 %) probably due to MDR with albendazol in all school children. Low HIV prevalence is probably result of outreach mobile HIV units and 5 years of voluntary counseling testing program (since 2008) as well as high proportion of patients on HAART due to five years of governmental program in South-East Uganda since 2006.

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MILITARY-TO-MILITARY ENGAGEMENT TO ENHANCE MALARIA PROGRAMS DURING PEACETIME AND DEPLOYMENT IN EAST AFRICA

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Malaria remains an important parasitic disease of public health concern, especially in Africa. Malaria is a problem for military forces because of its ability to cause sudden epidemics which can hinder or halt operations. During the Malaria Symposium hosted by the United States Africa Command in April 2011, representatives from several African militaries proposed formation of a multi-national Malaria Task Force to address common military malaria programmatic challenges. After assessing countries' current malaria activities, willingness to participate, perceived needs and expected outcomes, five nations of the East Africa Community (Burundi, Kenya, Rwanda, Tanzania and Uganda) united to hold the first East Africa Malaria Task Force (E-AMTF) meeting in December 2011. The mission of the E-AMTF is to strengthen and expand effective malaria programs and provide support for military personnel, their families and communities. The E-AMTF intends to assist national and regional malaria programs in harnessing the full potential of the armed forces as behavioral and social change agents. In preparation for the second E-AMTF meeting in Tanzania, gap analyses of the various malaria program components (Prevention, Diagnosis, Treatment, Surveillance and Human Resources/Capacity Building) during both peacetime and deployment were conducted. The process of critically evaluating their programs helped identify, document, and evaluate program requirements against current capabilities. Based on the urgency and impact on partner nations' military malaria programs, components were prioritized. Partner nations will take their accountability roadmaps and have a follow-up review meeting with key stakeholders to review, endorse and validate the roadmaps and define clear roles and responsibilities. This regional multi-lateral cooperation between the militaries of partner African nations, leveraging data-driven programmatic assessments of their malaria program needs, allow for the collaboration with US agencies' assets to enhance and develop malaria programs.

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OCCURRENCE OF MALARIA IS DECREASING WITH HIGHER ATTITUDE IN BURUNDI HIGHLANDS

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Altitude above sea can influence the spectrum of infective diseases, especially those like dengue fever or malaria and other mosquito-vector transmitted diseases. We have monitored occurrence of malaria and other tropical diseases in 4 rural Burundian hospitals working within the St. Elisabeth Tropical Program. Buraniro hospital is the lowest localized one in average height of 1280 m, Gasura health center is in 1550 m above sea level, Rutovu hospital is placed in 2065 m and Murago in 2663 m above sea. Overall size of all hospital is similar, those counting for 80 - 120 beds. In all four hospitals, the overall number of health consultation and gynecological consultation, number of malaria and other tropical cases are registered using form-filling. We evaluated incidence of malaria during December 2011 among all hospitals. During the December 2011, 9524 health consultations and 1465 hospitalizations were carried out in those hospitals. Lowest proportion of malaria during December 2011 was detected in Murago (606 cases per month, 47,6%) and highest it was in Gasura (1559 cases, 91,3%), then in Rutovu (732 cases, 81,2%) and Buraniro (4436 cases, 78,6%). Comparing to other types of consultations (gynecological, AIDS, other tropical diseases), malaria was the most frequent disease, even though some patients received more than one type of consultation. In this study we showed, that occurrence of malaria negatively correlates with altitude above sea and was lowest in Murango hospital placed above 2500 m ($P < 0,05$), where we have noted 606 malaria cases of which 499 (83,2%) were microscopically confirmed. Proportion of AIDS-consultations was lowest in remote hospitals of Rutovu and Murago where only few people are travelling to large cities or crowded places (such as Great Lake Tanganyika).

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AN ASSESSMENT OF THE MALARIA-RELATED KNOWLEDGE AND PRACTICES OF TANZANIA'S DRUG RETAILERS: EXPLORING THE IMPACT OF DRUG STORE ACCREDITATION

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In Tanzania drugs can be purchased from 2 types of retail outlets: Part I pharmacies and drug stores. Since 2005 Tanzania has been upgrading the approximately 7,000 drug stores to Accredited Drug Dispensing Outlets (ADDOs), involving dispenser training, introduction of record keeping and enhanced regulation. ADDOs are permitted to stock 49 prescription only medicines, including artemisinin-based combination therapies. Non-ADDO drug stores can officially stock over the counter medicines only, although many stock prescription only antimalarials. By the end of 2011 ADDO conversion was complete in 14 out of 21 regions, but limited information is available on their performance. Here we examine the malaria-related knowledge and practices of Tanzania's drug retailers, exploring variation between the different types of drug retailers. The data were collected as part of the AMFm Phase 1 Independent Evaluation, commissioned by the Global Fund to Fight AIDS, Tuberculosis and Malaria, which draws on methods developed by the ACTwatch group. We conducted a nationally representative survey of antimalarial retail outlets in Oct-Dec 2011. We randomly selected 49 wards, and interviewed all outlets stocking antimalarials. As Part I pharmacies were relatively rare these were oversampled by including all pharmacies in the districts - larger administrative units in which the selected wards were located. Interviews were conducted in 334 Part 1 pharmacies, 148 drug stores in ADDO regions, and 261 drug stores in other regions. We will present findings on outlet characteristics (number of staff, staff education and qualifications); staff knowledge (of first line antimalarial drug and its dosing); and antimalarials and malaria diagnostics (availability, retail prices, markups, sales volumes and wholesale sources). ADDO conversion is frequently cited as a model for improving retail sector drug provision but there is concern that the impact may be constrained by staff turnover and inadequate regulatory supervision. This study will provide important information to inform future policy on drug retailers in Tanzania and elsewhere in the region.

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EVALUATING THE COST-EFFECTIVENESS OF INTERMITTENT SCREENING AND TREATMENT (IST) COMPARED TO INTERMITTENT PREVENTATIVE THERAPY (IPTP) DURING PREGNANCY IN PREVENTING LOW BIRTH WEIGHT: A MODEL-BASED ANALYSIS

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Malaria during pregnancy is the leading preventable cause of low birth weight in many areas of sub-Saharan Africa. The current recommended intervention is to administer up to 3 doses of Sulphadoxine Pyrimethamine (SP) to pregnant women during antenatal clinic visits (IPT-SP) to clear any existing infection and protect against re-infection. However, with the emergence of SP resistance in many parts of Africa, alternative strategies to IPT-SP are currently being evaluated. One such alternative is intermittent screening and treatment (IST), whereby long-acting artemisinin combination therapy is administered to women with a positive rapid diagnostic test (RDT). By linking a model of the progression of *Plasmodium falciparum* malaria during pregnancy to the risk of low birth weight, we explored the impact and cost-effectiveness of IST and IPT-SP in areas with different transmission intensity and levels of SP resistance. Our results suggest that in areas where the parasite is still sensitive to SP, IPT will be more cost-effective than IST. This is due to the limited sensitivity of RDTs to detect low-grade infections and the additional cost of the RDT relative to SP. However, in areas of East Africa with high levels of SP resistance, our results suggest that a switch to IST would lead to a reduction in the burden of malaria-attributable low birth weight. Whether IST is also more cost-effective depends mainly on the difference in cost between SP and the chosen RDT and less on the level of transmission, the level of immunity acquired or the relative cost of the antimalarial provided to those with a positive test. For example we found that in areas where SP fails to clear infections in 35% of parasitaemic women, IST would be cost-effective provided costs associated with an RDT are below $1 per test. In summary, our results suggest, conditional on our model assumptions, that a switch of policy to IST would only be effective in reducing the burden of low birth weight in areas where there are moderate to high levels of SP resistance, with the degree of resistance necessary to make such a decision cost-effective depending primarily upon the cost of the RDT used.

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MALARIA RISK FACTORS IN UNDER-FIVES CHILDREN IN OUELESSEBOUGOU, MALI

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Childhood malaria is a major cause of mortality particularly in sub Saharan Africa, and we do not understand the intrinsic or acquired mechanisms of resistance. We have undertaken intensive longitudinal cohort studies to assess malaria risk factors and acquired protective immunity. A cohort of children aged 0-3 years of age was enrolled starting in September 2010. During the 2011 malaria transmission season (July to December), thick and thin smears were performed every two weeks in under-ones children, every four weeks in older toddlers, and also at the time of any illness. Clinical malaria was defined as the presence of asexual stages of *P. falciparum* on blood smear with signs or symptoms of malaria. Poisson regression was used to assess the relationship between host factors and clinical malaria risk. During the season, 486 malaria episodes occurred in 265/479 (55.3%) enrolled children (incidence rate of 1.03 episodes per child per season), with some children experiencing up to six clinical episodes in the season. The risk of clinical malaria was lower in children less than 2 years of age compared to those of 2-4 years (0.84 versus 1.2 episodes/child/season; adjusted incidence rate ratio (IRR) =0.70; 95% CI 0.58 - 0.84; $p < 0.001$). The risk was also significantly lower in children with hemoglobin S, (0.68 versus 1.07 episodes/child/season; IRR = 0.63, 95% CI 0.42 - 94, p =0.02). No significant association was found with blood group (ABO, Rh) or Fulani ethnicity. Preliminary analysis of this single season data indicates that hemoglobin S and age are associated with resistance to clinical malaria. Additional factors, such as iron status, are being assessed, and will provide a detailed definition of which subsets of children are or are not susceptible to malaria. This context will allow us to undertake detailed immunologic studies in the susceptible children, and define the targets and mechanisms by which children become resistant to clinical malaria.

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DEFINING THE MALARIA BURDEN IN NCHELENGE DISTRICT USING THE WHO MALARIA INDICATORS SURVEY

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Malaria is considered as one of the major public health problems and among the diseases of poverty. In areas of stable and relatively high transmission, besides children under 5 years of age, pregnant women and their new born babies are among the higher risk groups. A multicentre trial on the safety and efficacy of several ACTs during pregnancy is currently on-going in 4 African countries, including Zambia, whose study site is in Nchelenge district. As the study outcomes may be influenced by the local malaria endemicity, this needs to be characterised. Therefore, in March-April 2012 we carried out a cross-sectional survey to determine the prevalence and intensity of malaria infection among <10 years old children in Nchelenge district, on the shores of Lake Mweru. The sampling unit was the household where all children < 10 were included in the survey. We used a simple random selection of households using the GPS coded list. Individual consent to participate was collected from parents/guardians. A blood sample for Hb measurement and the detection of malaria infection was collected as well as information on the use of preventive measures such as Long-Lasting Insecticidal Nets (LLIN). Three hundred twelve households were sampled and 358 children included in the survey. Malaria parasite prevalence was 31.3% (95% CI: 26.6-36.4%); anaemia prevalence (Hb <11g/dl) was 49.1% (95% CI: 43.8-54.6%), a higher value than those previously found in the province. Though malaria has declined substantially in Zambia, there are still pockets of high endemicity such as Nchelenge district. These areas should be targeted for achieving high coverage of preventive interventions such as LLIN and indoor residual spraying.

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CHANGES IN MALARIA PREVALENCE AND HEALTH PROVIDER'S BEHAVIOR TOWARDS FEVER WITH THE INTRODUCTION OF ACT AND RDT AT PERIPHERAL HEALTH CENTRE LEVEL IN SOUTHWESTERN SENEGAL (2000-2011)

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During 2000-2011, the staggered introduction of ACT (artesunate-amodiaquine, ASAQ) and RDT in Mlomp (~6000 inhabitants), South-western Senegal coincided with profound changes in health providers' behaviour and malaria epidemiology. Through 2006 ASAQ and microscopy were rolled-out on experimental basis; from 2007 ASAQ+RDT were policy and free of charge. Injectable quinine has been available throughout. The dispensary is the only health provider in the village. The dispensary registries recorded 67,015 consultations, of which 35,169 (52%) for fever. Fevers accounted for 62% of consultations in 2000 vs. 33% in 2011; fevers dropped -74%, consultations -51%. Of all fevers, 9147 (26%) were diagnosed clinically as non-malaria (from 10% in 2000 to 88% in 2011) and treated accordingly, and 26,022 were clinically-suspected malaria (from 5046 in 2000 to 176 in 2011, -97%). The number of confirmed malaria fevers dropped by >90% from 1365 in 2000 to 112 in 2011) Of these, 23,481 (90%) received an antimalarial treatment (-36% in 2011 vs. 2000), of which 6893 (29%) were for parasitologically-proven malaria (P+), 10,122 (43%) for parasitologically-negative fevers (P-), and 6466 (28%) without a parasitologic diagnosis (P0). Overall, 18,859 clinically-suspected malaria underwent parasitologic confirmation (72%). No change was seen in any of the above. ASAQ accounted for 12% of antimalarial treatments overall (41% of treatments for P+, 7% P-, 9% P0). Comparing 2007-11 (ASAQ + RDT deployed) to 2000-06, the yearly number of fevers halved, non-malaria fevers doubled, malaria treatments dropped -86%. ASAQ increased from 17% to 30% of antimalarial treatments and from 57% to 94% of P+ cases. There was no difference in the proportion of fevers tested parasitologically (75% with microscopy during 2000-06, 70% with RDT during 2007-11), nor in the P.*falciparum* positive rate (29% vs. 31%). Case management of fever improved (better detection of non-malaria fevers, few malaria treatments). Practice compliance with malaria policy increased (almost all treatments are ASAQ), ca. three-quarters of fevers are tested parasitologically. However, introduction of RDTs did not boost testing significantly (presumably because of prior successful training in microscopy), and confidence in RDT is still limited (presumably because the proportion turning out positive is low compared to clinical suspicion - the established prior practice).

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RAPID DIAGNOSTIC TESTS AS A TOOL FOR MOLECULAR SURVEILLANCE OF *PLASMODIUM FALCIPARUM* MALARIA

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Prompt and accurate parasitological confirmation of *Plasmodium falciparum* malaria is essential for effective disease management. WHO recommends the use of malaria Rapid Diagnostic Tests (RDTs) in settings where microscopy services are not available. Following improved global malaria control and regional elimination efforts, there is a critical need for novel surveillance tools and strategies. Used RDTs have shown to be a reliable source of parasite DNA. Together with highly sensitive molecular assays, wide scale collection of used RDTs may serve as a modern tool for improved malaria case detection and drug resistance surveillance. The aim of this study was to compare and evaluate different methods of DNA extraction from RDTs and to test the field applicability for the purpose of molecular epidemiological investigations. DNA was extracted from two RDT devices (Paracheck-Pf and SD Bioline Malaria Pf/Pan), seeded *in vitro* with ten-fold dilutions of cultured 3D7 *P. falciparum* parasites diluted in malaria negative whole blood. The level of *P. falciparum* detection was determined for each extraction method and RDT device with multiple nested-PCR and qPCR assays. The field applicability was tested on 875 paired RDT (Paracheck-Pf) and filter paper (Whatman 3MM) blood samples collected from febrile patients in Zanzibar 2010. Preliminary *in vitro* results show that DNA extraction efficiency varied with extraction method and RDT device. The method of *P. falciparum* detection influenced the detection limit by 1-2 log units. No apparent difference in quality of DNA extracted from RDTs and filter papers was observed, in terms of PCR results from both *in vitro* and field samples. The results support the field applicability of RDT-DNA extraction for the purpose of improved molecular surveillance of antimalarial drug resistance, malaria case detection and RDT quality control.

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PLASMODIUM FALCIPARUM EXPOSURE SINCE BIRTH AND RISK OF SEVERE MALARIA: A NESTED CASE-CONTROL STUDY ON THE COAST OF KENYA

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Severe malaria affects mainly young children in *Plasmodium falciparum* endemic areas. The mechanisms by which immunity to severe malaria develops remain largely unclear, as does the number of infections needed to acquire protection. The aim of this study was to establish how exposure to *P. falciparum* infections during the first years of life affects the risk of severe malaria. A cohort of 5949 children born 2001-2008 in Kilifi District on the Kenyan Coast was followed with three-monthly visits from birth until 2 years of age. Infection patterns in children who subsequently developed severe malaria (according to strict criteria) were compared to three-monthly profiles of age-matched community controls in a 1:3 nested case-control design. Detection of *P. falciparum* by microscopy or PCR in at least one sample from birth conferred an increased risk of severe malaria and particularly if a multiclonal infection, as defined by genotyping of the polymorphic merozoite surface protein 2 gene, was ever detected. Antibodies to *P. falciparum* schizont extract were similarly prevalent in cases and controls, indicating the overall same level of exposure. In this area of moderate-low malaria transmission, parasite positivity and diversity since birth confer an increased risk of developing severe malaria. This study demonstrates for the first time with parasitological data differences in previous exposure between children who developed severe malaria and community matched controls.

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MEASURING *PLASMODIUM FALCIPARUM* TRANSMISSION IN LOW-ENDEMIC SETTINGS USING A COMBINATION OF COMMUNITY PREVALENCE AND HEALTH FACILITY DATA

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As some malaria control programs shift focus from disease control to transmission reduction, there is a need for transmission data to monitor progress. At lower levels of transmission, this becomes increasingly difficult to measure precisely, whether through entomological or other studies. Many programs conduct regular cross sectional parasite prevalence surveys, and have access to malaria treatment data routinely collected by ministries of health, often in health management information systems. By themselves, these data are poor measures of transmission. We propose an approach for combining annual parasite incidence and treatment data with cross-sectional parasite prevalence and treatment seeking survey data to estimate the incidence of new infections in the human population, also known as the force of infection, with limited supplementary data. The approach is based on extension of a reversible catalytic model. The accuracy of the estimates from this model appears to be highly dependent on levels of detectability and treatment in the community, indicating the importance of information on private sector treatment seeking and access to effective treatment.

904

SPATIAL AND TEMPORAL TRENDS IN MALARIA TRANSMISSION CAN BE CAPTURED BY THE DIAGNOSTIC POSITIVITY RATE REPORTED FROM SUMMARIES OF QUALITY ASSURED HEALTH FACILITY RECORDS RELAYED THROUGH MOBILE PHONES

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Measurement of malaria incidence among humans is central to monitoring malaria control program implementation. Quality-assurance and rapid reporting systems are required to reliably measure malaria transmission through passive reporting systems as current health management information system reports are not sufficiently rapid or reliable. Weekly summaries of malaria Rapid Diagnostic Test (mRDT) results from 14 health facilities (HFs) in Luangwa and Nyimba districts of central and eastern Zambia were reported via mobile phone text message. Diagnostic positivity rates reported by this passive monitoring system were compared with both the detailed data from the facility patient registers and a longitudinal incidence cohort comprising clusters of approximately 1000 residents in the immediate catchment areas of each facility. While passive HF-based surveillance reported fewer cases of malaria (10345 versus 12267, P=<0.0001), particularly non-febrile cases (33 versus 8311, P=<0.0001), the diagnostic positivity rates obtained correlated well with geographic (P=0.002) and temporal (P=0.000) heterogeneity in rigorously measured incidence rates. The HF surveillance system described adequately captured malaria transmission trends in local HF catchment populations and offers a cost-effective method for fine-scale program monitoring that can be applied on large scales. In conclusion, rapid, accurate reporting of quality-assured HF records of mRDT diagnostic positivity could enable population-wide, continuous longitudinal monitoring of malaria transmission so that integrated vector management programmes can be effectively managed, optimized by both local and national malaria control programmes.

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SEROLOGY CONFIRMS MODELED RISK FOR TRANSFUSION MALARIA FROM BLOOD DONORS WITH TRAVEL TO MEXICO AND AFRICA

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There have been only 7 cases of transfusion-transmitted malaria (TTM) reported in the US since 1998, most attributable to former African residents. The apparent efficacy of current US malaria policy in preventing TTM is counterbalanced by annual deferral of ~160,000 US blood donors for travel-associated malaria risk. Most deferred travelers have visited low risk areas, especially Mexico (66,000/yr), which recent models suggest presents malaria risk 1000x lower than Africa. We compared estimates of modeled risk with measured malaria risk based on antibody (Ab) testing of donors deferred for travel to Africa and Mexico. Blood donors deferred for malaria risk (travel/residency in an endemic country or past history of malaria) were recruited, consented and enrolled. Study subjects provided 2 EDTA tubes of blood and completed a risk-factor questionnaire. Samples were tested for *Plasmodium* Abs by EIA (Lab21 Healthcare); repeat reactive (RR) samples were considered positive and tested by real-time PCR. Since 2006, 6,077 deferred donors were tested by EIA, including 5,879 deferred for travel. Overall, 91 (1.5%) subjects were RR, with 49 (54%) reporting a history of malaria infection; none were PCR positive. Only two (0.2%) of 1,223 travelers to Mexico were RR, with both reporting prior infections acquired elsewhere (Turkey, 1976 & Ghana, 2005). Among 275 donors tested for travel to Africa, 9 (3.3%) were EIA positive, 6 reported a history of malaria; all 9 were infected in Africa. Travel to Mexico accounts for a large percentage of US donors deferred for malaria risk, but most visit low risk areas. Testing of travel deferred donors identified no cases of malaria acquired in Mexico, supporting modeled estimates of exquisitely low risk associated with travel to Mexico. In contrast, few donors are deferred for travel to Africa yet acquire infection at much greater rates from travel to or residence in Africa. A more effective approach to preventing TTM would be to defer donors reporting a past history of malaria or significant exposure in high risk areas (i.e., Africa).

906

MAINTENANCE OF UNIVERSAL COVERAGE OF LONG-LASTING INSECTICIDE TREATED BEDNETS (LLINS) IN RWANDA: PRELIMINARY RESULTS OF LONGITUDINAL LLIN DURABILITY AND EFFICACY STUDY

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The use of long lasting insecticidal nets (LLINs) is a proven effective malaria control intervention. While LLINs are expected to last for 3-5 years or 20 washes, the reality of net effective life in Rwanda could be different. Rwanda achieved universal bednet coverage (1 net per 2 people) in February 2011after distributing 6.1 million LLINs since 2009. In December

2010, Rwanda initiated a 3 year longitudinal study to track net efficacy in 3,000 LLINs (1500 polyethylene/permethrin; 1500 polyester/deltamethrin) at 6 sites. At one month post-distribution, and every 6-monthly interval, 10 LLINs are sampled from each site and tested for bio-efficacy (insecticidal effect) using WHO cone bioassay. A colorimetric field test (CFT) is used to assess surface deltamethrin levels. LLIN durability is assessed using a probability hole index (pHI) and theoretical cutoff values to identify the percentage of LLINs in good or serviceable condition. Preliminary results show that at one month, 6 months, and 12 months following distribution 8.5%, 12.9%, and 17.8% of LLINs were missing. LLIN cone bio-efficacy decreased to an average of 84.3% (84.0-84.7) at 6 months and 83.8% (83.3-84.3) at 12 months. Deltamethrin surface levels show 50-80% depletion of insecticide after 6 months with little change at 12 months compared to the baseline. LLINs remain viable with effective insecticide surface concentration at least equivalent to10% of the baseline. The durability assessment indicates that in 5 out of 6 sites <10% (pHI>768 threshold: polyethylene: 3-10%; polyester: 7-30%) of LLINs would require replacement after 6 months and 32% after 12 months (pHI>768 threshold; polyethylene: 37%; polyester: 13-50%). The data suggest that LLINs remain effective after one year of use in Rwanda. However, projected CFT and durability trends indicate that approximately 50% may become ineffective in the next 6 months. These observations highlight the need to conduct LLIN efficacy and durability studies to guide strategies for LLIN replacement and ensure effective universal coverage.

907

STUDY ON PERSISTENCE OF INSECTICIDES (BIOASSAY TEST) IMPREGNATED NET-JACKETS FOR MALARIA PREVENTION IN RUBBER TAPPER GROUP AT SURATHANI PROVINCE

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This study aimed to find out persistence of difference insecticides that were taken to impregnate net-jackets and to compare persistence of used and unused impregnated net-jackets in laboratory and field trial. The net-jackets were impregnated by insecticides namely Permethrin 10% EC, Deltamethrin 1% SC and Alphacypermethrin 10% SC at dosage 300 mg/m2, 30 mg/m2 and 30mg/m2, respectively. Impregnated net-jackets were tested at laboratory room temperature and some were given to rubber tapper volunteer group at Surathani Province in field trial. This volunteer group usually daily wore impregnated net-jackets while they had worked at night. Evaluation was conducted by bioassay test method that *Anopheles dirus* (laboratory stain) was tested to determine insecticide persistence. In laboratory trial, impregnated net-jackets were bioassay tested after impregnation 4, 8, 18 and 24 weeks. The result of persistence of three insecticides showed mortality rate of An. dirus that were more than 80% significantly at 24 weeks or 6 months. In field trial, impregnated net-jackets were bioassay tested after impregnation 2,4 and 8 weeks. The result of impregnated net-jackets could kill An. dirus effectively that were less than 4 weeks. Deltamethrin and Alphacypermethrin were higher effectiveness than Permethrin. Thus, persistence of unused impregnated net-jackets (Permethrin 300 mg/m^2, Deltamethrin 30 mg/m^2 and Alphacypermethrin 30 mg/m2) were more than 6 months at temperature room. The used impregnated net-jackets would have persistence at less than 4 weeks.

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STRENGTHENING COMMUNITY SYSTEM COMPONENTS FOR MALARIA CONTROL: FIVE YEARS INTERVENTIONS IN PASTORALIST COMMUNITIES IN AFAR REGION-ETHIOPIA

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A community based malaria prevention and control programme has been implementing in Afar Region since 2005. The goal of the programme was to contribute to the reduction of malaria related morbidity and mortality among pastoralist population in Afar region, by specifically targeting children under five years and pregnant women.Interventions focused on improving case management of malaria, increasing ITN coverage at community level, and enhancing behaviour and social change in support of all interventions. A baseline survey was done followed by a midline survey in 2007 and a final evaluation in 2010. Both quantitative and qualitative data collection methods were employed to collect data from respondents to assess the impact of this five years programme by comparing the key indicators before and after intervention. The proportion of community members who correctly identified the transmission methods of malaria had increased from 27.4% in 2005 to 88% in 2010. ITN coverage of at least one had significantly changed from 7.5% of base line year to 76% in year 2010. ITN usage of pregnant women and children under five who slept under ITN had also considerably increased from 27% to 79% and 17% to 82% respectively between the two periods.Furthermore, treatment seeking behavior was also improved and the percentage of children under five with fever who took antimalarial drugs within 24 hours increased from 9% at baseline to 53.4% at end of the evaluation period. Mortality rate at health facility level decreased dramatically from 25% in 2005 to 2% at the end of year 2010.The results indicate that strengthening community system in pastoralist populations and linking them to the health system improve the capacity of the community to own their health and contribute to reduce malaria mortality.

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MAN, ECOSYSTEM HEALTH AND MALARIA ON RUSINGA ISLAND, WESTERN KENYA

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Malaria is a leading cause of morbidity and mortality in Kenya. Existing evidence indicates that prevalence of the disease is greatly influenced by human and ecological factors. While it is well known that certain alterations on natural ecosystems aggravate malaria there is a dearth of information about the relationship between malaria and livelihoods. This study seeks to find the relationship between livelihoods, malaria and ecosystem health. The underlying objective is to generate an evidence base for reviewing malaria health and control policy. The study will be conducted on Rusinga Island, Lake Victoria, western Kenya, where local residents rely on fishing and small-scale farming to support their livelihoods. By carrying out fishing activities outdoors and at night Rusinga fishermen are exposed to a higher risk of malaria because (i) transmitting mosquitoes mainly bite at night, and (ii) currently deployed vector control tools are designed for indoor mosquito control. The apparently high risk of malaria posed by fishery and certain small-scale farming activities will be investigated. This will be accomplished by determining malaria prevalence and screening for mosquito saliva antigens among individuals associated with different livelihood practices. The specific livelihood-related groups of people that will be recruited for studies include subsistence farmers, fish traders, boat owners, fishing crew and stakeholders involved in fishery activities namely net/boat makers/repairers, transporters, and fish bait miners/traders. We will also attempt to explain how actions directed towards supporting livelihoods modify ecosystems in ways that may aggravate malaria. Communication tools will be developed to share knowledge generated from these activities among the local residents. An outcome mapping model will be developed to measure changes in behaviour among collaborating boundary partners namely Kibisom women group and the Mbita district public health office (Mbita-DPHO).

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SIMULATED COMMUNITY-LEVEL EFFECTS OF COMBINING LONG LASTING INSECTICIDAL NETS WITH INDOOR RESIDUAL SPRAYING FOR MALARIA CONTROL IN AFRICA

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It is common practice to combine indoor residual spraying (IRS) with long-lasting insecticide nets (LLINs) in highly endemic communities, but there is limited evidence to suggest that the strategy confers greater protection against malaria than either intervention alone. Experimental hut trials have demonstrated improved personal and household protection with certain LLIN/IRS combinations, but it remains unclear whether there are proportionately greater benefits at community level. A deterministic mathematical model of mosquito life cycle processes was adapted and used to estimate how malaria transmission might be affected if LLINs are combined with IRS, relative to use of either method alone. The model was modified to use data derived directly from experimental hut evaluations where untreated bed nets are used as experimental controls. We simulated a closed community where residents own cattle, and the main malaria vector is *Anopheles arabiensis*, an increasingly important vector species in Africa, which remains a major challenge even with high LLINs and IRS coverage. Considering situations with either LLINs or IRS as the pre-existing intervention, we calculated relative improvement in transmission control each time a complementary intervention was introduced. Transmission control is improved when the common pyrethroid based LLINs are added onto toxic IRS treatments such as pirimiphos-methyl and lambda cyhalothrin, but not DDT, which is known to be less toxic against mosquitoes. On the other hand, the outcome remains unchanged when IRS with lambda cyhalothrin or DDT is added to communities already using LLINs. Addition of pirimiphos-methyl IRS provided the greatest improvement relative to the LLINs alone. This *in-silico* assessment shows that whereas introduction of LLINs into communities with pre-existing IRS will generally result in improved control of malaria transmission, introduction of IRS into communities with pre-existing LLIN use will most likely be redundant unless the IRS is highly toxic to malaria mosquitoes.

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EVALUATING PROGRESS OF INTENSE IMPORTED MALARIA TRANSMISSION IN SOUTH AFRICAN PROVINCES: RETROSPECTIVE ANALYSIS

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For the past 50 years, because of national and global malaria strategies put in place vector transmission was low in South Africa. Successful early malaria control policies and strategies developed non-immunity to malaria amongst most South Africans. The results were an increased risk of complicated and severe infections from *Plasmodium falciparum* and other untreated vector species. Southern African populations consistently visit or migrate to and from malarious areas, including countries bordering South Africa. Exposure to mobile populations with malaria infections contributes to the burden of disease in South African Provinces. The most vulnerable are children under five, pregnant women and those with co morbidities such as HIV and TB. Imported malaria is identified as a major concern within endemic and non endemic Provinces of South Africa in regional mapping and by the Republic of South Africa's National Malaria Programme Performance Review-2009. A geographical focus is used to identify high transmission areas in South African Provinces in low lying North East areas of: Limpopo, KwaZulu-Natal and Mpumalanga where malaria is endemic and seasonal. A literature review synthesized previous research from 1982-2012. The Study also analyzed trends in the understanding and knowledge of imported malaria in Southern Africa. Quantitative indicators are used to build on existing malaria control measures in South and Southern Africa while evaluating the progress of the intense burden of imported malaria in South Africa. This was achieved by analyzing: sentinel surveillance measures, malaria control interventions, and transmission rates based on data from mosquito breeding sites and climate. The Study emphasizes sustainable capacity building for: surveillance, quantification and local community participation. Cross border malaria initiatives from five countries bordering the Provinces of: Limpopo, KwaZulu-Natal and Mpumalanga were analyzed based on the quantitative indicators described. Studying imported malaria in South Africa is a regional and global contribution to: improving surveillance, human interaction with ecological systems that are breeding sites for mosquitoes, economic development, health outcomes and public health policies associated with malaria as a debilitating and potentially fatal infection.

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IMPACT OF TREATING *PLASMODIUM FALCIPARUM* ASYMPTOMATIC CARRIERS WITH ARTEMETHER-LUMEFANTRINE ON MALARIA TRANSMISSION

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Asymptomatic *Plasmodium falciparum* carriers serve as parasite reservoirs for malaria transmission; and community screening and treatment of these asymptomatic carriers with artemether-lumefantrine (AL) may reduce the pool of infectious gametocytes and potentially malaria transmission in that area. To test this hypothesis, entomological parameters were assessed before and after AL treatment in a longitudinal entomological survey. Mosquitoes were collected once per week using the indoor spray catch method in 5 villages where mass screening and treatment of asymptomatic carriers was implemented, and in 5 control villages. Data collected were used to infer the human-biting rates. Mosquitoes collected were processed by ELISA assay to estimate sporozoite index and entomological inoculation rate (EIR) in each site and susceptibility of malaria vectors to insecticide was also determined. The population was provided with long-lasting insecticide treated nets (ITNs). A total of 15768 mosquitoes were collected during the survey. After morphological identification, 90% were confirmed as *Anopheles gambiae* s.l. The remaining catch were not *Anopheles*. The temporal analysis shows that the density of mosquitoes increases from January to August, which is mainly due to the rainy season creating an abundance of breeding sites. The comparison of vector composition, human-biting rates, EIR and insecticide susceptibility between the control and the intervention zones is done to measure the impact of the intervention on the malaria transmission. Results will be presented at the conference.

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DEVELOPMENT OF LABORATORY TESTS FOR THE PHYSICAL DURABILITY OF LONG-LASTING INSECTICIDAL BEDNETS

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A key performance attribute of long-lasting insecticidal nets (LLINs) is durability. According to the WHO, $3.8 billion could be saved between 2011 and 2020 if LLIN longevity can be increased from 3 years to 5 years. Although technical advances in LLIN longevity have focused on insecticide retention, there is growing evidence that the net fabric can also deteriorate rapidly in many settings. As insecticide resistance becomes a greater concern, the ability of LLINs to maintain physical integrity during years of use becomes increasingly important. Despite this, no laboratory test method yet exists to evaluate the physical durability of LLINs and researchers must conduct multi-year comparison studies of LLINs in the

field to acquire durability data. The objective of the present work is to develop one or more laboratory tests that can be used to evaluate how well LLINs withstand realistic physical challenges, using standard textile testing equipment. The focus has been on measuring the susceptibility of fabrics to deterioration after suffering initial damage by rodents or hot surfaces. Modifications of standard bursting strength, tensile strength, tear resistance, and abrasion resistance test methods have been evaluated for reproducibility and consistency with the results of field studies. This investigation has also provided insight into the mechanisms of LLIN deterioration and possible strategies for improving durability.

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MODELING THE EFFECTS OF VECTOR CONTROL INTERVENTIONS IN REDUCING MALARIA TRANSMISSION AND DISEASE BURDEN

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Malaria interventions are usually prioritized using efficacy estimates from intervention trials, without considering the context of existing intervention packages or long term dynamics. Currently, long lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) form the mainstay of most malaria control programs. However, in the face of emerging resistance in mosquitoes and a push to elimination, extensions and new combinations of these interventions are being considered, along with the development of novel interventions, such as outdoor traps, and a revival of older interventions such as larval source management. We use numerical simulations of an ensemble of mathematical models of malaria in humans and mosquitoes to provide robust quantitative predictions of the effectiveness and cost-effectiveness of combinations of these interventions, in reducing transmission, morbidity and mortality. We estimate reductions in entomological inoculation rate, prevalence, clinical cases, and malaria deaths from simulations of different coverage levels of LLINs, IRS, larval control, and outdoor traps. We simulate scenarios with various vector distributions, and transmission and health system settings. Our results suggest that sustained coverage of one or two vector control interventions reduces malaria prevalence through the first two or three campaigns but does not lead to continually increasing gains beyond that. However, in some settings, even with sustained coverage, clinical incidence of malaria increases as the population loses its naturally acquired immunity. In some low to medium transmission settings, our simulations suggest that high coverage of both LLINs and IRS can lead to interruption of transmission; however, larval control or outdoor traps are necessary when a separate population of mostly outdoor biting mosquitoes exists. We can simultaneously capture in mathematical models the dynamics of mosquito ecology, malaria epidemiology, human demography, health systems effects, and control interventions. Fitting an ensemble of models to data leads to plausible quantitative predictions, with accompanying uncertainty ranges, of the effects of a comprehensive set of different interventions in reducing and potentially interrupting transmission.

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MARKET COMPETITION AND CUSTOMER DEMAND DETERMINE STOCKING PATTERNS AND RETAIL PRICES OF ACTS IN PRIVATE DRUG SHOPS IN TANZANIA

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Many sub-Saharan African households utilize the private sector as a primary source of treatment for malaria episodes. Cost, however, is reported to be a major impediment to shop level stocking of effective anti-malarials such as ACTs. The Affordable Medicines Facility - malaria (AMFm), an innovative financing mechanism hosted by the Global Fund, is a unique supply side subsidy designed to increase availability of ACTs in eight pilot countries including Tanzania. A series of shop level surveys of private drug retailers in two regions of Tanzania aimed to discover supply and demand side factors which determine availability and reported retail sale price of subsidized ACTs in private shops. Accredited Drug Dispensing Outlets (ADDOs) in the Rukwa and Mtwara regions were surveyed between Feb 2011 and May 2012. Surveyors noted whether subsidized ACTs were being sold and recorded retail prices. Shop attendants were asked a battery of questions including malaria knowledge, source of drug supply, and participation in training programs. Exit interviews with customers at the ADDOs provided data on the type and price of the antimalarial purchased. Trends and extent of ACT stocking over time and space were described using statistical and spatial methods. Supply side determinants of ACT stocking were assessed using distance to reported medicine supplier. Competition from surrounding providers was assessed using the location of retail shops and public reproductive health clinics (RCH). GIS layers for surrounding population were used to estimate the size of catchment populations. Remoteness and distance to wholesale sources of medicines were not found to be associated with ACT stocking patterns. Shop size and population were found to be highly associated with ACT stocking. Proximity to other shops stocking ACTs (p=.04) and increased number of competing shops (p=.006) were statistically significantly associated with an increased likelihood of stocking ACTs in Rukwa. Similarly, presence of RCH clinics determined stocking. High numbers of proximal shops was also associated with increased prices charged for ACTs. Stocking of ACTs and prices charged were both higher in areas along Lake Rukwa than in other areas of similar distance from urban areas and population size. The AMFm program appears to have resulted in increased availability of ACTs, though shop level factors also influence stocking and prices charged.

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INDUCIBLE INSULIN-LIKE PEPTIDE SYNTHESIS IN *ANOPHELES STEPHENSI*: A MECHANISM FOR *PLASMODIUM* MEDIATED IMMUNOSUPPRESSION

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The insulin-like peptides (ILPs) and their respective signaling and regulatory pathways are highly conserved across diverse phyla. Previously, we reported that infection with the human malaria parasite, *Plasmodium falciparum*, induces ILP transcription in the midgut of *Anopheles stephensi*, suggesting that the ILPs are produced in response to infection-associated signals and modulate some aspects of sporogonic development. In particular, our data revealed that soluble factors derived from *P. falciparum*, but not from bacteria or fungi, can induce ILP transcription and secretion in *An. stephensi* cells. This induction was dependent on insulin/insulin-like growth factor signaling (IIS) through MEK-ERK and PI3K-AKT activation. Additionally, knockdown of an infection-induced ILP *in vivo* resulted in enhanced immune effector gene expression and decreased parasite survival in *P. falciparum* infected mosquitoes. Together, these data suggest that *Plasmodium*-specific factors signal through IIS to induce immunosuppressive ILPs in the midgut, a critical tissue for parasite development. The ILPs should be considered, therefore, important targets in future efforts to engineer *Plasmodium*-resistant mosquitoes.

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AGE AND MALARIA RISK DETERMINE INSECTICIDE TREATED NET USE NEAR LAKE VICTORIA, MBITA DISTRICT, KENYA

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Despite scaled-up coverage of insecticide treated nets (ITNs) in malarious areas of sub-Saharan Africa, proper and regular ITN use remains inadequate. An understanding of what determines ITN use could help improve effectiveness. In early 2011, a household-level, questionnaire-based survey of ITN practices was conducted following a mass distribution program. The goal was to assess post-intervention *Plasmodium* infection, and whether households used ITNs to protect target groups such as pregnant women and children. Following a complete enumeration of all households (~3,340), each one was censused for all residents and surveyed. Pre-school children were tested for presence of parasites using PCR methods. Questions of household heads involved who slept under what ITN the previous night, as well as age and sex. Data analysis involved spatial methods and regression models tailored to account for non-linear patterns in age-related ITN use. GIS methodologies were used to determine spatial patterns of ITN use and malaria cases. Information on 12,095 individuals aged 90 years old was gathered, of which ~25% were <5 years of age. More than half (56%) of people reported not sleeping under an ITN the previous night. Age was an important determinant of ITN use. Adults over 30 and infants sleep under ITNs more than children and young adults. The distribution of age and ITN use followed a significant ($p < 0.001$) nonlinear pattern, decreasing from birth to age 18, increasing to and remaining constant after age 30. This pattern was significant even when accounting for confounding factors. Differences in gender were not significant for any age group, but women between the ages of 15 and 30 tended to use ITNs more than males. Household-level clusters of *Plasmodium* infections were associated with fewer children sleeping under nets, and were geographically located in wet, low lying areas closer to the lake, despite high levels of net use and possession. Though ITNs were found to be effective in reducing *Plasmodium* infections, spatially, evidence suggests that net possession and use were highest in areas prone to nuisance mosquitoes and possible perception of high malaria risk. Results suggest that ITN use may be high among some members of higher-risk groups, however there is inadequate coverage among young and school-age children. Efforts to further scale up ITN possession and programs to focus messages regarding proper use remain necessary

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SHIFTING FROM BLANKET TO TARGETED INDOOR RESIDUAL SPRAYING FOR MALARIA CONTROL IN ZANZIBAR: A NOVEL APPROACH FOR INTEGRATED MANAGEMENT OF MALARIA VECTORS

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Zanzibar (1.2 million population) has significantly reduced *Plasmodium falciparum* prevalence to less than 1% over six years through scale-up of multiple malaria interventions, including indoor residual spraying (IRS) and long-lasting insecticidal nets (LLINs). Between 2006 and 2011 six rounds of blanket IRS with lambda-cyhalothrine were applied to 210,000 structures. A policy to transition from blanket to targeted IRS was agreed to by Ministry of Health and partners in 2009 pending universal coverage of LLINs, establishment of a weekly surveillance system for diagnostically confirmed malaria cases, and an insecticide resistance mitigation plan (IRMP). In 2012 universal coverage of LLINs was achieved, weekly malaria surveillance was scaled-up to all 142 public health facilities with complete (100%) and timely (77% by Friday) reporting, and an IRMP was introduced. Malaria incidence was calculated for each health facility catchment area as the number of confirmed *P. falciparum* cases per 1000 population per year and used as the primary indicator for selecting locations for targeted IRS. Incidence for all of Zanzibar in 2011 was 2.4/1000/yr (95% CI, 2.3-2.5/1000/yr). The peak transmission period incidence was 5.6/1000/yr (95% CI, 5.4-5.8/1000/yr) during May to August compared to 0.7 and 0.9/1000/yr during January to April and September to December, respectively. We defined three risk strata: A) <0.3 cases/1000/yr; B) 0.3-15 cases/1000/yr; and C) >15 cases/1000/yr. Category A received no targeted IRS, category B one round, and category C two rounds. Based on the seasonal incidence data, the first targeted round of IRS with bendiocarb was completed in March 2012 for 120,000 structures (category B and C). A second round will target 15,000 of these same structures (category C) in September 2012. Zanzibar has met policy prerequisites to transition from blanket to targeted IRS. Weekly surveillance data will be monitored to assess whether targeted IRS can further reduce malaria transmission. These findings will help inform other malaria control programs considering a scale-down IRS after universal LLIN coverage is achieved.

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STRENGTHENING COMMUNITY SYSTEM FOR MALARIA CONTROL: THE CONTEXT OF GLOBAL FUND GRANT IN SENEGAL

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Community involvement in health programming aims to achieve improved outcomes of interventions to deal with major health challenges such as HIV, tuberculosis and malaria. This is vital for making progress towards universal access to health care and meeting the Millennium Development Goals. Support for community-level and NGO programming is a key component of Global Fund grants. However, there remains lack of evidence and lessons learned about how Community System Strengthening (CSS) can be developed, effectively implemented and linked to the formal health system in a malaria endemic country. This paper reports Senegal's National Malaria Control Programme experience on CSS using Global Fund opportunity. Available information generated between 2005 and 2010 from the program database, annual reports, reports to the Global fund, partners reports, program performance review reports, surveys and published articles were reviewed. The Global Fund framework for CSS was used to analyze the malaria program contribution. Sixty nine District Health Teams (DHTs)- through District Community Networks Against Malaria- and 16 NGOs were involved as sub recipients to implement community based interventions. A total of 34,628 community volunteers were trained to carry out sensitization and awareness campaigns, distribute nets, and destroy breeding sites. A further 3,176 community health workers (in the health huts) and 861 Home Care Providers (HCPs) in remote areas were involved in malaria case management using RDT and ACT. In this process, local capacity was built and stakeholders involved in the entire process from planning to assessment. ITN coverage increased among children under five from 9.7% in 2005 to 45% in 2010. Between 2009 -2010, 12,582 suspected malaria cases were managed by HCPs, 93% of whom were tested with an RDT. Among those tested, 37% had a positive RDT, 97% of whom were treated and got cured. CSS by building capacity of local communities and actively involving them in improving their own health is a key means to control malaria and sustain gains in resource poor countries.

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IMPACT OF COMMUNITY SCREENING AND TREATMENT OF ASYMPTOMATIC CARRIERS OF *PLASMODIUM FALCIPARUM* WITH ARTEMETHER-LUMEFANTRINE ON ASYMPTOMATIC AND GAMETOCYTE CARRIAGE: A 12-MONTH, CLUSTER-RANDOMIZED STUDY IN SUB-SAHARAN AFRICA

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Human to mosquito transmission of *Plasmodium falciparum* depends on the presence of sexual stage parasites, gametocytes, in the peripheral blood. Interventions in asymptomatic carriers (ACs) aiming to reduce disease transmission should also be effective against gametocytes. This 12-month, controlled, parallel, cluster-randomized (18 clusters: 9 intervention, 9 control) study was conducted in Burkina Faso to evaluate the impact at the community level of systematic screening and artemether-lumefantrine (AL)/AL dispersible treatment of RDT-detected ACs during three community screening campaigns (CSCs 1-3). CSCs 1-3 occurred before the rainy season and CSC4 occurred after, marking the end of the study. Symptomatic malaria episodes were treated with AL or an alternative in both arms during the study. The prevalence of microscopy-confirmed ACs in the intervention and control arms was 42.8% vs. 47.5%; 4.1% vs. 35.7%; 2.8% vs. 32.2% and 34.4% vs. 37.8% at CSC1, 2, 3 and 4, respectively. The proportion of gametocyte carriers (GCs) was evaluated by microscopy in all subjects at CSCs 1-4 in the intervention arm and in a randomly selected 40% subset of the control arm, and by qRT-PCR at CSC4 in 1,999 randomly selected subjects across both arms. The overall proportion of GCs in the intervention and control arms was 9.5% vs. 10.2%, 0.6% vs. 5.5%, 0.4% vs. 5.8% and 4.8% vs. 5.1% at CSC1, 2, 3 and 4, respectively. The prevalence (least square mean (SE)) of microscopy-confirmed GCs at CSC4 in the intervention arm was 4.9 (0.41) vs. 5.1 (0.41) in the control arm (p=0.7208). Prevalence of GCs at CSC4 as assessed by qRT-PCR was around 8 times greater in both arms compared to microscopy (49.7% vs. 6.0% intervention; 47.3% vs. 5.4% control). In this community-setting study, the intervention arm showed greater reductions in the prevalence of ACs and GCs than the control arm at CSCs 2 and 3, relative to CSC1 (p<0.0001). However, AC and GC prevalence rose thereafter in the intervention arm to reach a level similar to the control arm at CSC4 (p=NS).

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ONLINE INTERACTIVE PLATFORM FOR MAPPING REPORTS OF INSECTICIDE RESISTANCE IN MALARIA VECTORS

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Insecticide-based interventions including indoor residual spraying and treated bed nets have led to significant reductions in malaria morbidity and mortality. However, the emerging and rapid spread of resistance to all available classes of public health insecticides threatens current malaria vector control efforts. The Global Plan for Insecticide Resistance Management released by the WHO in May 2012 contained guidance on the rationale and implementation of strategies for preserving the efficacy of current tools, which included utilization of insecticide resistance data for informing vector control decisions. There has long been a need for a comprehensive global resistance database to aggregate data currently scattered across many sources in order to facilitate a coordinated response across the malaria-stakeholder community. IR Mapper was developed to address this need (www.irmapper.com); this free online resource consolidates published information from WHO susceptibility tests on *Anopheles* malaria vectors from 1959 to date. Information is provided via a user-friendly interface that allows users to project data on maps based on selected vector species, insecticide classes and types. Susceptibility data are viewable based on old WHO susceptibility categories or using the new categories as recommended from May 2012. Resistance mechanism data are similarly presented, with links to original data sources provided along with other key study information. The utility of this resource will be demonstrated using examples from two large-scale malaria control programs in Africa.

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SEROPREVALENCE OF TOSCANA VIRUS INFECTIONS AMONG RURAL POPULATIONS IN AZERBAIJAN

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The sandfly fever viruses are endemic to the Mediterranean region and well known causes of human illness there. The Toscana virus (TOSV) serotype displays a unique neurotropism and can cause a meningitis or meningoencephalitis in some cases. Little is known about the geographic distribution of Toscana virus outside of the Mediterranean basin. While the *Phlebotomus* spp. sandfly fever vector has been associated with visceral leishmaniasis in Azerbaijan, the presence of TOSV infection in this region has never been assessed. In Azerbaijan, the etiology of febrile illnesses and cases of meningitis are underreported due to low healthcare utilization and lack of laboratory diagnostics. However, public health officials suspect high rates of arboviral infections along the Azerbaijan-Russia border. Previous studies have attempted to document the seroprevalence of several zoonotic infections in this region, however no studies to-date have evaluated the prevalence of TOSV infection there. We obtained 755 serum samples that were previously accessed from adults from the villages of Guba, Gusar, and Xachmaz as part of a cross-sectional study [USAMRIID HUC FY07-31 (APS TNK 004)(WRAIR 1435)] and analyzed the prevalence of TOSV antibodies using an IgG TOSV detection kit [DIESSE, Siena, Italy]. The subject population included 796 adults over the ages of 18 years, 72% of who were less than 50 years of age, 56.4% of whom were female, and 75.3% of Azerbaijani ethnicity. We found a total of 18 positive samples, resulting in a seroprevalence of 2.38%. This initial study is the first to document the presence of TOSV infection in the Republic of Azerbaijan. Although the majority of TOSV infections are asymptomatic, further testing may be warranted for suspected cases in which subjects present with fevers, headaches, and/or symptoms of meningitis or meningoencephalitis of unknown etiology. This study also helps to establish a need for further epidemiologic and surveillance studies in this region.

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DETECTION OF EASTERN EQUINE ENCEPHALOMYELITIS VIRUS RNA IN NORTH AMERICAN SNAKES

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The role of non-avian vertebrates in the ecology of Eastern Equine Encephalitis virus (EEEV) is unresolved, but mounting evidence supports a potential role for snakes in the EEEV transmission cycle, especially as over wintering hosts. To determine rates of exposure and infection, we examined serum samples from wild snakes at a focus of EEEV in Alabama for viral RNA using RT-PCR. Two species of vipers, the Copperhead

(*Agkistrodon contortrix*) and the Cottonmouth (*Agkistrodon piscivorus*), were found to be positive for EEEV RNA using this assay. Prevalence of EEEV RNA was more frequent in seropositive snakes than seronegative snakes. RT-PCR positivity in cottonmouths peaked in April and September. Body size and sex ratios were not significantly different between infected and uninfected snakes. These results support the hypothesis that snakes are involved in the ecology of EEEV in North America, possibly as winter maintenance hosts for the virus.

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A LABORATORY CONFIRMED CASE OF JAMESTOWN CANYON VIRUS ENCEPHALITIS IN A QUEBEC RESIDENT WITH TRAVEL HISTORY TO MAINE AND NEW HAMPSHIRE

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Jamestown Canyon virus (JCV) is a mosquito-borne arbovirus belonging to the California serogroup (CSG) of bunyaviruses. JCV is widely distributed throughout North America, however, reports of human JCV infection with associated febrile and neurological disease are rare. We report a recent laboratory confirmed case of JCV encephalitis in a Montreal, Quebec resident with travel history to Maine (ME) and New Hampshire (NH). The patient was a 53 year old male who presented with symptoms of fever, headache and chills 10 days after returning from a camping trip in ME and NH in mid August, 2011. Several days later he was hospitalized and his illness progressed to an altered mental state comprising of confusion and difficulty speaking suggestive of encephalitis. He had trouble breathing and was intubated. He was hospitalized for approximately a month during which blood was collected and lumbar punctures performed. Cerebrospinal fluid (CSF) testing indicated normal protein and glucose with the presence of a low leukocyte count. Serological testing of acute and convalescent serum collected three weeks apart gave a 4-fold rise in specfic neutralizing antibody to JCV (640 to 2560). Using a CDC-based IgM ELISA the acute and convalescent serum samples were positive for JCV IgM. Testing of acute and convalescent CSF for JCV antibodies also indicated a positive IgM result for JCV and a seroconversion by neutralization testing (range 0 -16). Significantly lower or negative cross reacting titres were observed for related CSG viruses such as snowshoe hare and La Crosse viruses and no antibodies were detected to other arboviruses such as West Nile, eastern equine encephalitis, or Powassan virus. Based on several laboratory case definition criteria our results indicate that the patient's febrile and neurological symptoms were associated with an infection of JCV. The incubation period of JCV is believed to range from 3 to 14 days and the travel history of the patient is consistent with exposure to JCV infected mosquitoes in ME or NH. It is also possible that he may have been infected in Quebec since symptom onset did occur several days after his return to Canada. Based on available CDC CSG virus case data no confirmed cases of JCV illness have been documented in ME or NH previously. Our findings underscore that JCV can cause serious neurinvasive disease such as encephalitis and should be considered when an arbovirus infection is suspected in this region of North America.

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VIRAL ETIOLOGIES OF DIARRHEA AMONG CHILDREN ATTENDING LWAK MISSION HOSPITAL IN ASEMBO, WESTERN KENYA

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Enteric viruses are important causes of gastroenteritis in young children globally. Most etiologic studies in developing countries have focused on hospitalized children with a paucity of outpatient cases. We characterized the etiologic distribution, epidemiology and clinical characteristics for diarrhea-associated viruses in children less than fourteen years of age living in a rural area in western Kenya. Two-hundred and six stool specimens collected between January 2007 and June 2010, from children ≤14 years old with diarrhea, who visited the study clinic for population-based infectious disease surveillance in Asembo, were screened for enteric viruses. Enzyme immunoassays were used to detect rotavirus and adenovirus, and reverse transcriptase multiplex polymerase chain reaction (RT-PCR) assay was used for norovirus, astrovirus and sapovirus. At least one viral agent was detected in 26.7% (55/206) of specimens; rotavirus was detected in 13.6% (28/206), norovirus in 6.3% (13/206), adenovirus in 4.9% (10/206), astrovirus in 2.9% (6/206) and sapovirus in 1.5% (3/206) respectively. Viral co-infection was shown in 9.1% (5/55) of positive specimens, with 4 of 55 co-infections attributable to rotavirus dual infections. Ages of the children ranged from 3 months to 14 years (mean age = 4.6 years; median = 2.5 years). About one third (32.6%) of the specimens with a virus detected were from children less than 2 years of age. The main clinical symptom of the children from whom a virus was detected was fever (78.6%). These findings suggest that at least five enteric viruses are potentially important agents for diarrhea in this rural site in western Kenya. Defining clinical and epidemiologic characteristics predictive of viral etiology may have implications for the management of diarrhea in children in Kenya and similar settings.

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IDENTIFICATION OF NEUTRALIZING ANTIBODY EPITOPES ON CHIKUNGUNYA VIRUS ENVELOPE PROTEIN

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To obtain anti-Chikungunya (CHIKV) Envelope monoclonal antibody (MAb) epitope maps at the resolution of individual amino acids, we individually mutated 920 residues of CHIKV (S27 strain) Envelope protein (E2/E1) to alanine, expressed each mutant in human cells, and analyzed them for effects on antibody reactivity and viral infectivity. This 'Shotgun Mutagenesis' approach offers the capability of mapping both linear and conformational epitopes, even for structurally complex proteins including oligomeric and glycosylated Envelope proteins such as CHIKV E2/E1. The neutralizing human anti-CHIKV MAbs used in our studies were derived from infected patient B-cells using phage display library panning against purified CHIKV virus like particles (VLPs) and from B-cell cloning. Critical amino acids required for the binding of each MAb were identified and visualized on the E2/E1 protein structure. We also determined the binding affinity and kinetics of these MAbs to intact CHIKV VLPs on a biosensor. Our goal is to map epitopes on CHIKV Env protein, determine how they

contribute to neutralization of infection, and how they relate to protein function. We expect that this approach will help define the range of immunodominant structures on CHIKV Env and identify novel neutralizing antibody epitopes that can be used for the development of improved therapeutics, diagnostics, and vaccine candidates.

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DEVELOPMENT OF AN IRES-BASED VACCINE FOR WESTERN EQUINE ENCEPHALITIS VIRUS

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Western equine encephalitis virus (WEEV), is a member of the family Togaviridae, genus Alphavirus, has a single-stranded, positive-sense RNA genome, and is an important mosquito-transmitted human and veterinary pathogen in North and South America. Infection with WEEV can result in severe neurological sequelae in human survivors, with an economic impact ranging from 21,000 to 3 million dollars per case. WEEV is also considered a bioterroism agent since aerosolized virus causes high primate mortality. Regrettably, there is no vaccine or antiviral therapies to aid in mitigating a natural outbreak, bioterrorist attack, or accidental lab exposure. The objective of this study was to develop a safe and efficacious WEEV vaccine. Two different live-attenuated WEEV vaccines were engineered via the introduction of an internal ribosomal entry sequence (IRES) from encephalomyocarditis virus (EMCV), to control translation of the structural (WEEV/IRESv1) or capsid (WEEV/IRESv2) protein(s). Previous research shows the IRES element from EMCV cannot initiate efficient translation in arthropod cells, making this vaccine unable to be propagated in its natural transmission cycle. Serial passaging in Vero cells showed no reversion to a wild-type-like phenotype; however, several mutations were observed in the structural genes that provided for higher titers in cell culture. WEEV/IRESv1 and WEEV/IRESv2 were tested for immunogenicity and attenuation in relevant murine models. Our results suggest that our IRES-dependent live-attenuated vaccine for WEEV merits further study and this vaccine could be used for the development of an emergency vaccine that can be used during a natural outbreak, bioterrorism attack, or accidental lab exposure.

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DISTRIBUTION OF KILLER CELL IMMUNOGLOBULIN-LIKE RECEPTORS (KIR) GENES IN AN ADMIXED PERUVIAN POPULATION

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KIR are glycoproteins located on the surface of NK cells. These receptors are classified into two groups according to their cytoplasmic domain, which transduces inhibitory or activating signals, and consequently modulates NK cell function and most likely the susceptibility to diseases or infections. We studied the distribution of KIR genes in 363 Peruvian HTLV-1-infected individuals using two ethnic classification methods: 1) a questionnaire, which defined the participants as Andean (both parents born in the Andes) or Mestizo (only one parent born in the Andes); and b) ancestry informative markers (AIM), which allowed classifying the whole population into three groups according to their ethnic admixture proportions. DNA was obtained from blood samples of each individual and KIR genotyping was carried out using PCR-SSP. No significant differences were observed in gender and age according to the Andean/Mestizo classification, whereas significant differences were found when the ethnic admixture proportion criterion was applied. The frequency of KIR2DS3, KIR2DS4 and KIR2DL3 were statistically different between Andeans and Mestizos. When using ethnic admixture proportion, significant differences were observed for KIR3DL1 and KIR2DS4s in addition to those genes, among the three groups defined. No significant differences were detected in haplotypes and inhibitory-activating KIR genes using either the questionnaire or AIM-based classification. AIM helps minimizing both the bias in ethnic group definition and the effects of population stratification, and therefore should be used in order to avoid false results when searching for gene-disease associations in admixed populations.

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NOROVIRUS INFECTION IN PERU

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Norovirus (NoV) is one of the most frequent causes of outbreaks and sporadic cases of gastroenteritis worldwide. Although rarely fatal, NoV transmission has important economic repercussions, including loss of work days and incurrence of costly medical care. The incidence of NoV gastroenteritis is usually highest in adults. Although well-studied in industrialized countries, few data are available on NoV in the developing world. We report NoV surveillance data from 3 distinct regions and populations in Peru. The first two surveillance populations and areas were healthy children <5 years old in rural communities near the town of Pisco (coastal desert) in 2009 and in Loreto District (Amazon forest) in 2010. The third target population and surveillance area was from a 9-year prospective cohort study of diarrheal illness among Peruvian military recruits at the Vargas-Guerra Army Training Base in the city of Iquitos, also in the Amazon. At each site, fecal samples were collected and sent to the U.S. Naval Medical Research Unit-6 laboratory in Lima for testing for NoV by real-time PCR. From Pisco, NoV was found in 27 (9%) of 294 samples. Five (19%) were genotype I and 22 (81%) II. From Loreto, 32 (11%) of 290 samples were positive_10 (31%) genotype I and 22 (69%) genotype II. From the Vargas-Guerra Training base, 49 (25%) of 200 samples were positive_6 (12%) for genotype I, 38 (78%) for genotype II, and 5 (10%) co-infection with both genotypes. Our results indicate that NoV circulates in both pediatric and adult populations in Peru and that genotype II predominates. Interestingly, infection was common even in the healthy children. Epidemiologic studies are underway to explore the significance of the NoV infections in children and the ultimate incidence of disease. In addition, we are undertaking further molecular characterization and phylogenetic analysis of NoV strains in Peru.

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SEROPREVALENCE OF ALPHAVIRUSES AND FLAVIVIRUSES IN FREE-RANGING GAME ANIMALS AND NON-HUMAN PRIMATES IN THE CONGO BASIN

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Vector-borne and zoonotic pathogens have comprised a significant proportion of the emerging infectious diseases in humans in recent decades. The role of many wildlife species as reservoirs for arthropod-borne viral pathogens is poorly understood. We aimed to investigate the exposure history of various African wildlife species from the Congo Basin to mosquito-borne flaviviruses (*Flaviridae: Flavivirus*) and alphaviruses (*Togaviridae: Alphavirus*) by testing previously-archived serum samples. In total, sera from 24 African forest buffalo (*Syncerus caffer nanus*), 34 African elephants (*Loxodonta africana*), 40 duikers (*Cephalophus* and *Philantomba* species), 25 mandrills (*Mandrillus sphinx*), 32 mountain gorillas (*Gorilla beringei beringei*), five Grauer's gorillas (*Gorilla beringei graueri*), two L'hoest's monkeys (*Cercopithecus lhoesti*), two golden monkeys (*Cercopithecus kandti*), and three chimpanzees (*Pan troglodytes*) sampled between 1991 and 2009 in the Congo basin were tested for antibodies against chikungunya virus (CHIKV) (*Togaviridae: Alphavirus*),

o'nyong-nyong (ONNV) (*Togaviridae: Alphavirus*), West Nile virus (WNV) (*Flaviridae: Flavivirus*), dengue 2 virus (DENV-2) (*Flaviridae: Flavivirus*), and yellow fever virus (YFV) (*Flaviridae: Flavivirus*) by plaque reduction neutralization test. Specific neutralizing antibodies against ONNV were found in African forest buffalo in the Democratic Republic of the Congo (DRC) and Gabon, duikers in the DRC, and mandrills in Gabon, providing novel evidence of enzootic circulation of ONNV in these countries. In addition to ONNV, African forest buffalo in the DRC and Gabon had been exposed to CHIKV, WNV, and DENV-2, while mandrills in Gabon were also seropositive for CHIKV, DENV-2, WNV, and YFV. One hundred percent of elephants tested had a very strong neutralizing antibody response to WNV. This study also represents the first arbovirus serosurvey of gorillas, of which 4/32 (12.5%) were seropositive for either an alphavirus and/or flavivirus. Our results demonstrate a high prevalence of neutralizing antibodies against these arboviruses in wildlife in the Congo basin.

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SIMIAN FOAMY VIRUS AND HERPES VIRUS IN CAPTIVE NEW WORLD PRIMATES IN PERU

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Many species of New World primates (NWPs) exist in Peru and are frequently illegally captured for pet trade, traditional medicine, or consumption. Government confiscation and placement of these animals has led to extreme crowding in zoos and rescue and rehabilitation centers, providing ideal conditions for animal-human transmission of zoonotic pathogens. Simian foamy viruses (SFV) are retroviruses found in high prevalence in various simian species. Infection has occurred in humans exposed to Old World monkeys and apes in captivity and in nature. Previous reports show genetically distinct SFV variants among NWPs but these data are limited to small numbers of captive monkeys from genera *Cebus, Saimiri, Ateles*, and *Callithrix*. Herpes viruses are ubiquitous agents that infect a variety of animals, with co-evolution between each unique herpes virus and its reservoir species. Herpes viruses are easily transmitted from their reservoirs to accidental hosts through direct contact and fomites, often resulting in a fatal outcome. We assessed the prevalence of SFV and herpes virus infection in captive NWPs to help assess the risk of infection of these zoonotic viruses to humans with exposure to these animals. Serum samples were collected and tested for antibodies to SFVs by enzyme immunoassay, with confirmatory Western blot analysis (antibody in retroviruses correlates with active virus infection), and for herpes virus by PCR. Sixty (38%) of 157 tested NWPs were antibody positive for SFV, including animals from the genera *Alouatta, Aotus, Ateles, Callicebus, Callithrix, Cebus, Lagothrix, Pithecia, Saguinus* and *Saimiri*. Twenty-one (15%) of 144 tested NWPs were PCR positive for herpes virus. Molecular characterization of the viruses is ongoing to identify the specific viruses and, in the case of the herpes viruses, whether they are human or NWP species. Our results show a high prevalence of SFV and herpes viruses in captive NWPs in Peru. We plan follow-up studies to explore the prevalence of human infection to these viruses among Peruvians in contact with captive NWPs.

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FULL LENGTH SEQUENCING AND GENOME ASSEMBLING STRATEGIES OF DISTINCT ARBOVIRUSES USING PYROSEQUENCING DATA

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The current work describes different strategies to obtain complete genomes for arboviruses. A total of 176 arbovirus isolates (20 Mayaro, 30 dengue-1, 30 dengue-2, 30 dengue-3, 18 dengue-4, 30 Oropouche, 12 yellow fever, 3 ungrouped arboviruses and 1 of each Guama, Catu, and Bimiti viruses) were cultured in VERO cells or newborn mice and used as source for obtaining the RNA. Supernatant of infected VERO cells or mice brain suspensions were pre-treated with DNAse/RNAse for removing of host DNA/RNA contaminants. Non-treated and pre-treated suspensions were then submitted to the RNA extraction. For dengue, Oropouche, and Mayaro viruses a set of specific primers were designed to produce large amplicons (including UTR and ORFs) through a long amplification using commercial kits. Large amplicons were gel purified, and used for sequencing using the pyrosequencing method. For unknown viruses, the shot-gun methodology was applied for generation of random viral complementary genomic fragments. The genomes were assembled using distinct software (Newbler, Mira and Geneious pro) and methodologies (reference mapping and *de novo* assembling strategy). A computational pipeline was created and automated to remove all possible host contaminant sequences before viral genome assembling. Regardless the nature of the RNA (segmented or non-segmented), the complete sequences were obtained for all 176 isolates. The reducing of host contaminant DNA/RNA increased substantially the number of reads for a given virus, as well as the genome coverage. Comparisons among non-treated and pre-treated samples revealed that pre-treated samples increased the viral genome coverage in 40% in size and in 50% in depth. A mean of 120 x coverage was reach and few gaps were found within the ORFs. Gaps were closed by specific Sanger sequencing. Mostly of the uncovered regions corresponded to the UTRs which were recovered using the RACE strategy. Three computationally algorithmic were used improving the genome assembling closing eventual gaps. The dengue, Oropouche, Mayaro and yellow fever virus isolates had their genomes confirmed using the Blast algorithmic; the results for unknown viruses have revealed two different rhabdoviruses and one orthobunyavirus. The combination of sample pre-treatment, long RT-PCR or *de novo* sequencing, and bioinformatic analyzes represent powerful tool for rapid sequencing, assembling and identification of known and new arboviruses.

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HERD IMMUNITY AND POTENTIAL VACCINE IMPACT ON OUTBREAKS OF HAND FOOT AND MOUTH DISEASE IN SOUTHEAST ASIA

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Hand Foot and Mouth Disease (HFMD) is typically a mild and self-limiting childhood infection caused by any number of viruses in the Enterovirus genus of the Picornaviridae family, with the most commonly implicated

pathogens being Coxsackievirus A16 (CA16) and Enterovirus 71 (EV71). Increasingly, outbreaks of HFMD, particularly those caused by EV71, have garnered the attention of the public, clinicians and national and international health agencies. Recently, there has been an alarming increase in the number of patients and an increase in the number of cases complicated by central nervous system and cardiopulmonary involvement and deaths in young children in countries across South East Asia. Epidemic patterns are complex, though large outbreaks tend to be cyclical, occurring every 2 to 3 years. With vaccine in development, the question remains whether HFMD caused by CA16 or EV71 is an immunizing infection and whether infection by one pathogen confers protection from infection by the other. Using over a decade of weekly infection case data from Japan and Singapore, we find that the case data are consistent with the pathogen acting as an immunizing infection with a strong signature of herd immunity. Preliminary simulations indicate that vaccinations could succeed at limiting epidemics. We validate these findings with multiannual infection case data from other countries in the region. Our finding that HFMD acts qualitatively as an immunizing infection is promising for the success of future vaccination efforts for the control of this disease. Further surveillance and regional cooperation including analysis of epidemiological, clinical, and virological data sets for Hand Foot and Mouth Disease would help guide future control strategies and inform policy.

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HANTAVIRUS RODENT RESERVOIRS IN BULGARIA

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Hantaviruses are a group of RNA viruses belonging to the Bunyaviridae family, genus Hantavirus, and their natural reservoirs are wild rodents. Hantaviruses are the global emerging diseases, with evolving strains detected throughout the world. Different serotypes as Seol (SEO), Dobrava (DOB), Pumuula (PUU) and Hantaan (HTN) were detected in the Balkan region. Human disease causes from DOBV in Bulgaria were recently confirmed. In a previous study on hospital-based acute febrile illness cases, we detected PUUV IgM using ELISA (Progen) and DOBV neutralizing antibody by PRNT. In this study we conducted rodent surveillance in areas with documented human cases to identify the circulating strains of Hantaviruses. Rodents were trapped starting May 2011 through March 2012 for two successive night patterns per month. Temperature and humidity were monitored at specific GPS coordinates recorded for trapping sites. A total of 705 rodents from 2 different sites (Plovdiv and Burgas) were trapped. Fourteen different rodent species were collected during the study period. Serum samples and organs (lung, spleen and kidneys) were collected. An organ pool, preserved with RNA-later, was homogenized under BSL3 conditions, followed by RNA extraction using Qiagen products. Real-time RT-PCR for DOBV and PUUV testing was performed on rodents collected in May, June and October (n=224), of which three DOBV and no PUUV position samples were detected. All DOBV was found in male *Apodemus flavicollis* species (susceptible to DOBV) collected in June: two from Plovdiv and one from Burgas. Screening of the remaining rodent collection is still in progress, and analysis of temporal conditions and abundance of species may provide a potential outbreak prediction model.

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EVALUATION OF VIRUS STRAINS AND THE EFFECT OF E1 MUTATIONS ON THE EXPRESSION OF CHIKUNGUNYA VIRUS-LIKE PARTICLES

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A challenge to the development and manufacture of affordable vaccines for a global market is reducing the cost of goods required for vaccine production. Due to the inverse relationship between the productivity of antigen expression and cost, increasing the efficiency of antigen production is one strategy used to reduce cost. In collaboration with the NIH, we are developing a chikungunya virus (CHIKV) vaccine based on transient expression of ORF2 in human cells to produce CHIKV virus-like particles (VLPs), as reported previously. In order to minimize the cost of goods, we have tested two strategies to increase the levels of CHIKV VLPs in culture supernatants. First, we evaluated the levels of CHIKV VLPs among 10 different CHIKV strains. Expression of strain 37997 ORF2 yielded the highest levels of CHIKV VLPs in the culture media, which was consistent with the study by Akahata, *et al.* Six strains including LR2006 OPY-1 and S27 exhibited low levels of VLPs, while two strains, ALSA-1 and Nagpur, failed to produce any detectable VLPs. Western blotting demonstrated a defect in p62 processing and low levels of E1 compared to strain 37997. As an alternate strategy for increasing VLP productivity, we tested the hypothesis that increasing the stability of the E2-E1 heterodimer by reducing the threshold pH for conformational changes in E1 that lead to membrane fusion may result in increased CHIKV VLP production. To test this hypothesis, we introduced 3 different E1 mutations that were shown to decrease the pH of fusion of another alphavirus. We found that those mutations increased the levels of VLPs in culture media after transient transfection. Moreover, additional substitutions for each of those 3 residues have identified several other mutants with enhanced VLP productivity. Current efforts are underway to determine if combining the mutations will further increase CHIKV VLP productivity. Work is also in progress to better understand the effect of these mutations on the stability of the E2-E1 heterodimer.

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MOLECULAR CHARACTERIZATION OF ANTIVIRAL SUSCEPTIBILITY OF INFLUENZA A ISOLATES OBTAINED IN KENYA FROM 2008 TO 2011

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Presently, there are two main classes of antivirals in use which function by inhibiting specific steps within the virus replication cycle: M2 inhibitors block the uncoating of the virus through acidification of the interior of the virion. In neuraminidase inhibition, inhibitor molecules mimic NA's natural substrate and bind to the active site, preventing NA from cleaving host cell receptors and releasing new virus. The study characterized antiviral susceptibility of the 2008-2011 influenza A strains using known molecular markers in neuraminidase (NA) protein. In the 2008-2009, 2009-2010 and 2010-2011 influenza seasons, a total of 836 viruses were isolated. 344 (41%) were influenza A/H3N2, 144 (17%) seasonal influenza A/H1N1 and 348 (42%) belonged to the pandemic influenza A/ H1N1 strain. A total of 108 (13%) isolates were analyzed for susceptibility to NA inhibitors. In the year 2008, 33 influenza A/H3N2 and 11 seasonal influenza A/H1N1 were included in the genotypic characterization assay for neuraminidase inhibitor resistant mutations. Sequence assembly and alignment revealed absences of molecular markers of neuraminidase inhibitor drug resistance (Y275) in influenza A/H3N2. 64% (7) of the 2008 seasonal influenza A/

H1N1 isolates had resistant marker H275Y. 4 (36%) of the seasonal A/ H1N1 isolates, lacked the drug resistant marker depicting sensitivity to the class of drugs. Genetic analysis of the 48 pandemic influenza A/ H1N1 strains in 2009 showed that all were sensitive to oseltamivir through possession of histidine at position 274 of the neuraminidase protein sequence. The same pattern was duplicated in 2 of the pandemic influenza A/ H1N1 isolates analyzed in the year 2010. All the 2011, 14 isolates belonging to influenza A/H3N2 subtype lacked the H275Y substitution in the neuraminidase protein. Genotypic data obtained in this study demonstrate antiviral resistance in seasonal influenza A/H1N1 viruses isolated in Kenya in 2008-2009 through possession of H275Y (N1 numbering) marker in the neuraminidase protein.

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APPLICATION OF *IN VIVO* IMAGING IN THE CHARACTERIZATION OF OLFACTORY INFECTION OF MICE WITH WEEV

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Isolates of western equine encephalitis virus (WEEV) can cause severe disease in both humans and animals, and may serve as a model for other neurovirulent alphaviruses. Infection of the McMillan strain (McM) of WEEV leads to high mortality in an outbred CD-1 mouse model. An infectious recombinant WEEV.McM expressing firefly luciferase (FLUC) was developed to characterize CNS infection after intranasal exposure. Correlative relationships were determined between bioluminescence and both viral titer and immunological markers of WEEV.McM/FLUC. Histopathological examination of tissue was guided by corresponding images and revealed that neuroinvasion occurred primarily through the olfactory tract. Olfactory bulb neurons were initial targets and led to the infection of the anterior olfactory nucleus, basal ganglia, hypothalamus, amygdala, thalamus, hippocampus, and cerebrum. IHC staining showed intense neurotropism with very few supportive cells infected. Neuronal processes were highly stained for FLUC expression and presented patterns consistent with dissemination of virus through neuronal connectivity. Immunopositive axons were often seen in areas connecting immunopositive foci, even when foci were separated by substantial distances. An additional route of neuroinvasion through the trigeminal nerve pathway was observed and resulted in significant reporter expression within the brainstem (pons). Although recombinant virus was observed to be attenuated when compared to wild-type virus in both replication kinetics and induction of immunological markers of disease, manifestation of disease was comparable. Therefore, we feel that this system provides a quantifiable determination of WEEV infection. This may prove beneficial to future assessments of antiviral strategies aimed at treating disease arising from olfactory infection with New World alphaviruses.

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SEVERITY OF ACUTE RESPIRATORY INFECTIONS ASSOCIATED WITH RESPIRATORY SYNCYTIAL VIRUS, GUATEMALA, 2008-2012

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Respiratory syncytial virus (RSV) is a major cause of acute respiratory infections. The epidemiology of RSV in all age groups has not been well described in Central America, particularly regarding disease severity. We aimed to address these knowledge gaps with surveillance data from Guatemala. We conducted active surveillance of ambulatory visits due to influenza-like illness (ILI: cough or sore throat and measured fever >38°C) and hospitalizations due to acute respiratory infections (ARI: sign of infection and a respiratory sign or symptom) in Santa Rosa (Nov 2007-Mar 2012), Quetzaltenango (Feb 2009-Mar 2012), and Guatemala City (Nov 2009 - Apr 2011). Nasopharyngeal swab specimens were tested for RSV using real-time reverse-transcription polymerase chain reaction. Among ARI cases, we measured associations between RSV-positivity and indicators of severity using linear and logistic regression, adjusted for age, gender, surveillance site, and year. To test for effect modification by age, we added an interaction term for RSV-positivity and age <5 years to the models. We enrolled and tested for RSV 7919 patients; 5626 met the ARI and 2293 the ILI case definitions. In persons <5 years of age (n=5009), the proportion of cases RSV-positive was higher among ARI (34%) than ILI (17%); in person ≥5 years of age (n=2910), the proportions were similar for ARI and ILI (7%). Among ARI, RSV-positivity was associated with lower oxygen saturation (-0.9; 95% CI: -0.4, -1.4) and lower odds of admission to intensive care unit (OR=0.7; 95% CI: 0.6, 0.8), mechanical ventilation use (OR=0.70; 95% CI: 0.54, 0.91), and death in hospital (OR=0.69; 95% CI: 0.48, 1.00). We found a lower OR for death associated with RSV in persons <5 years of age (p=0.017). RSV infection is more common among hospitalized ARI compared to ILI cases in young children and ARI patients present with lower oxygen saturation if they are RSV-positive, both which suggest RSV is associated with more severe disease. However, other findings suggest RSV-positive cases are less severe. Further analysis is required to understand whether RSV infection causes more severe disease than other pathogens.

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TACKLING A GLOBAL CHALLENGE ON DOMESTIC GROUND: GEOGRAPHIC AND DEMOGRAPHIC ANALYSES OF TB IN ORANGE COUNTY, CA

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Orange County, California carries one of the country's highest burdens of tuberculosis (TB) at a rate of 6.4 cases per 100,000 population in 2009. In this generally affluent county marked by pockets of poverty, the sociodemographic makeup of the populace was discerned through the use of GIS technology. We carried out a retrospective cohort study of all TB cases diagnosed in Orange County, CA from 2005 to 2009 and of all TB hospitalizations from 2005 to 2008 and performed geographic and demographic analyses on the data. Based on global trends, we expected the burden to be highest in the poorest portions of the county as well as in those with high immigrant populations. We found the highest incidences of both TB cases and TB hospitalizations in the city

of Santa Ana. As the poorest city in the county, Santa Ana (per capita income $16,891), had a case rate of 9.65 per 100,000 population. The highest case rate by city was found in Westminster (23.76 per 100,000 population). Foreign-born patients treated by the county represented 85.4% of all TB cases, placing the burden largely on the immigrant population of the county. There were clear relationships between relative risks & case rates and per capita income of the city [R= 0.423 and R= 0.434, respectively]. Relative risk ratios indicated that males [1.49], Asians [8.55], and seniors (65 yrs+) [3.34] were at greatest risk for a TB infection. The relative risk for a TB infection in an Asian male aged 65 years or older was 21.6. At greatest risk for hospitalization for a TB infection: males [1.43], Asians [4.17], and seniors [4.52]. The relative risk of an Asian male aged 65 years or older in the county being hospitalized due to TB was 17.4. More than 71% of all TB hospitalizations were government-funded with total charges exceeding $29.4 million over 4 years. These data suggest that the burden of TB in Orange County warrants continued attention and additional resources and also demands a change in policy with regards to the domestic handling of global health issues.

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INDIVIDUAL LEVEL RISK FACTORS FOR SECONDARY TRANSMISSION OF INFLUENZA-LIKE ILLNESS: SECONDARY DATA ANALYSIS FROM THE BANGLADESH INTERRUPTION OF SECONDARY TRANSMISSION OF INFLUENZA STUDY (BISTIS)

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Respiratory infections are a leading cause of mortality worldwide. Understanding risk factors for secondary transmission to close contacts will facilitate development of interventions to prevent respiratory pathogen transmission. We describe index case-patient and susceptible contact risk factors for secondary transmission of influenza-like illness (ILI) in the control arm of a randomized controlled trial evaluating the impact of handwashing promotion on ILI in Bangladesh. We identified index case-patients with ILI (fever in persons < 5 years old, and fever with cough or sore throat in persons ≥5 years old). Susceptible contacts were persons without respiratory symptoms at enrollment living in household compounds of index case-patients. Compounds included index case-patient households and≥1 secondary households. We recorded demographics and behaviors among all contacts, and frequency of interaction with index case-patient in a subset. We conducted daily ILI surveillance from the day after enrollment to 10 days after resolution of index case-patient symptoms. We used logistic regression to evaluate risk factors for ILI, adjusting standard errors for clustering of illness in households and compounds. In compounds of 185 index case-patients, 1477 (91%) of 1615 contacts enumerated were susceptible and took part in surveillance. We detected 111 (8%) secondary cases of ILI. Index case-patient demographics were not associated with ILI in contacts. Contacts with ILI were more frequently < 2 years old (OR=7.5, 95% CI=3.6 – 15.9), and 2 to < 5 years old (OR=4.7, 95% CI=2.6 – 8.6) than contacts without ILI. After age group adjustment, reported frequent daily interaction with the index case-patient was the only significant individual-level risk factor: OR_{adj}=1.9, 95% CI=1.1 – 3.5. Sex, parental relationship, living in same household or sleeping in same room as index case-patient, smoking, and time spent in cooking space were not associated with ILI overall or in age group-stratified analyses. In this low-resource setting, young age and frequent interaction with ill persons were significant risk factors for susceptibility to household ILI transmission. These data underscore the need to distance young children from persons ill with respiratory symptoms. Studies should assess feasibility and efficacy of distancing between household members as a strategy to minimize transmission of respiratory infections to the most vulnerable.

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A COMMUNITY RANDOMIZED CONTROLLED TRIAL OF AN INTEGRATED HOME-BASED INTERVENTION IMPROVING HOUSEHOLD-AIR POLLUTION, DRINKING WATER QUALITY AND HYGIENE IN RURAL PERU

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Diarrhoea and acute lower respiratory infections are leading causes of childhood mortality. Simple low-cost interventions have proven efficient in reducing diarrhoea and severe pneumonia; however, an integrated package provides opportunities for synergism. We conducted a community-randomised controlled trial in 51 rural communities in Peru to evaluate an environmental home-based intervention package (IHIP) in reducing acute lower respiratory infections, diarrhoeal disease and preventing malnutrition in children under 36 months of age. All homes used open fires and 80% had access to piped, untreated water supplies. *E.coli* was found in drinking water in 66% of the households. The proportion of stunted children was 55%. In the intervention arm an improved stove (OPTIMA) was installed and members were trained in the correct use and proper maintenances; a solar disinfection of drinking water (SODIS) method was established; and a water faucet with a kitchen sink was installed and handwashing practices were promoted. Diarrhoea, respiratory (weekly) and anthropometric (every two months) surveillance was done at home during a 12 months period. To reduce potential impact of non-blinding bias, the control arm received a psychomotor stimulation programme, We randomized 51 communities and enrolled 534 children. Baseline characteristics were balanced between study arms: The rate of diarrhoeal episodes in children in the intervention was 2.8 episodes per child per year as compared to 3.1 episodes in the control arm. The relative rate was 0.78 (95%CI: 0.58-1.05). Similarly, care takers in the intervention group reported fewer days of diarrhoea (mean 4.9 vs. 6.4 days per year; OR: 0.71, 95% CI: 0.47-1.06). No effect on acute lower respiratory infections or child's growth rates was observed. In conclusion we found no evidence for synergistic effects associated with the intervention package. Introducing several interventions and messages simultaneously may have overwhelmed the households and compromised use, operation and maintenance of all components.

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ACTIVE HOUSEHOLD-BASED SURVEILLANCE AND REGIONAL VARIATION IN INCIDENCE OF INFLUENZA IN PERU

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Most estimates of the disease burden of influenza rely on passive sentinel surveillance at health clinics and hospitals. These estimates lack population denominators necessary for calculations of incidence, especially of milder disease, potentially leading to underestimates of the true burden. In 2009 we implemented active community-based household surveillance in 4 ecologically distinct regions of Peru: coastal desert (Lima), dry

forest (Tumbes), highlands (Cuzco) and rainforest (Puerto Maldonado). Approximately 7200 people in 1500 randomly selected households are visited 3 times a week. Nasopharyngeal swabs are taken from persons with influenza-like illness (ILI) and tested for influenza virus by RT-PCR. Here we report the incidence and seasonal patterns for the first 1.5 years (June 2009-December 2010) comprising 9344 person-years (py) of follow-up. The overall influenza incidence was 14/100py, with a 95% confidence interval (CI) of 13-15. Of these, 6.5/100py (CI 6-7) sought care, 1/1000py (CI 0.5-2) required hospitalization, and one died. Although surveillance started after the peak of the pandemic, the observed incidence was still higher in 2009 (16/100py, CI 15-18) than 2010 (13/100py, CI 12-14). The highest incidence was observed in ages 6 months-12 years (31/100py, CI 29-33), followed by 13-17 years (17/100py, CI 15-20), <6 months (13/100py, CI 7-25), and >17 years (6/100py, CI 6-7). Tumbes consistently showed the highest incidence (19/100py, CI 17-20), followed by Lima (17/100 py, CI 15-18), Puerto Maldonado (12/100 py, CI 11-13), and Cuzco (9/100 py, CI 8-10). The proportion of ILI due to influenza was 18% in ages <5 years, 46% in ages 5-17 years, and 30% in persons >17 years. Peak incidence was June-December in Lima and Cuzco and September-January in Tumbes and Puerto Maldonado, with a second peak in Tumbes during June-July. We estimate that 3.8 million people in Peru had influenza in 2009-2010. Furthermore, incidence and temporal patterns vary significantly by region and will require region-focused prevention and control strategies.

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INCIDENCE OF HUMAN METAPNEUMOVIRUS IN RURAL AND URBAN POPULATIONS IN KENYA, 2006-2011

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Human metapneumovirus (hMPV) is a suspected cause of acute respiratory tract infections, mostly in young children, the elderly and immunocompromised patients. We investigated the incidence of hMPV in Kenya using longitudinal, population-based surveillance in two sites; two villages (total population = 28,000) in Kibera urban slum located in Nairobi City together comprising one site and in thirty-three villages in a rural Asembo community (total population = 26,000) in western Kenya as the second site. Between 1st October 2006 and 24th March 2011, nasopharyngeal and oropharyngeal swabs collected from consenting patients meeting the case definitions for either hospitalized severe acute respiratory infection or outpatient influenza like illness were tested for hMPV by real time Reverse Transcription polymerase chain reaction (RT-PCR). Incidence rates were calculated as the number of hMPV cases by person-years of observation (pyo) per site with adjustments for patients meeting the case definition at study clinics who were not swabbed and for participants who sought medical attention at non-study clinics. The HIV status was included in analysis for patients aged >18 years. Seventeen (n = 17) hMPV isolates were sequenced at the 347bp F-gene fragment for subtyping. Of 9000 cases tested from both sites, 614 (6.8%) were positive for hMPV, consisting of 345/4284 (8.1%) in Kibera and 269/4716 (5.7 %) in Asembo. In Kibera, the adjusted rates were highest in children < 12 months [99.6/1000 pyo (95% CI 80.9 - 122.6)] and lowest in those >50 years: [0.7/1000 pyo (95%CI 0.1 - 5.3)]; in Asembo, the adjusted rates were highest in children aged 12 - 23 months [62.7/1000 pyo (95%CI 62.6 - 62.8)] and lowest in those aged in 18 - 34 years [14.6/1000 pyo (95%CI 14.5 - 14.7)]. In Kibera, 33% (14/43) of the hMPV-positive patients ≥ 18 years were also positive for HIV whereas in Asembo, 40% (12/30) of hMPV positive cases were positive for HIV. The clinical symptoms associated with hMPV included fever, cough, and runny nose. On genetic analysis, 5 of 17 (29 %) Kenya viruses belonged to subgroup A, and 12 (71%) viruses to subgroup B. No A1 subgroup viruses were detected. Thus hMPV incidence was higher in children aged ≤5 years in both study sites and incidence rates decreased with increasing age.

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ROLE OF TEMPERATURE, HUMIDITY AND RAINFALL ON INFLUENZA TRANSMISSION IN GUATEMALA, EL SALVADOR AND PANAMA

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Worldwide, seasonal influenza causes about 500,000 deaths and 5 million severe illnesses per year. The environmental drivers of influenza transmission are poorly understood especially in the tropics. We aimed to identify meteorological factors for influenza transmission in tropical Central America. We gathered laboratory-confirmed influenza case-counts by week from Guatemala City, San Salvador Department (El Salvador) and Panama Province from 2006 to 2010. The average total cases per year were: 390 (Guatemala), 99 (San Salvador) and 129 (Panama). Meteorological factors including daily air temperature, rainfall, relative and absolute humidity (RH, AH) were obtained from ground stations, NASA satellites and land models. For these factors, we computed weekly averages and their deviation from the 5-yr means. We assessed the relationship between the number of influenza case-counts and the meteorological factors, including effects lagged by 1 to 4 weeks, using Poisson regression for each site. Our results showed influenza in San Salvador would increase by 1 case within a week of every 1 day with RH>75% (Relative Risk (RR)= 1.32, p=.001) and every 1°C increase in minimum temperature (RR=1.29, p=.007); but it would decrease by 1 case for every 1mm-above mean weekly rainfall (RR=0.93,p<.001) (model pseudo-R^2=0.55). Within 2 weeks, influenza in Panama was increased by 1 case for every 1% increase in RH (RR=1.04, p=.003), and it was increased by 2 cases for every 1°C increase of minimum temperature (RR=2.01, p<.001) (model pseudo-R^2=0.4). Influenza counts in Guatemala had 1 case increase for every 1°C increase in minimum temperature in the previous week (RR=1.21, p<.001), and for every 1mm/day-above normal increase of rainfall rate (RR=1.03, p=.03) (model pseudo-R^2=0.54). Our findings that cases increase with temperature and humidity differ from some temperate-zone studies. But they indicate that climate parameters such as humidity and temperature could be predictive of influenza activity and should be incorporated into country-specific influenza transmission models.

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RANDOMIZED, DOUBLE-BLINDED, PHASE 2 TRIAL OF WR 279,396 (PAROMOMYCIN AND GENTAMICIN) FOR THE TREATMENT OF CUTANEOUS LEISHMANIASIS CAUSED BY *LEISHMANIA PANAMENSIS*

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This randomized double-blinded Phase 2 trial included 30 patients (17 adults and 13 children ages 5 to 17 years) with Leishmania panamensis cutaneous leishmaniasis (CL). Patients were randomly allocated (1:1) to receive once daily topical treatment with WR 279,396 (15% paromomycin + 0.5% gentamicin) or Paromomycin Alone (15% paromomycin) for 20 days. Patients were followed for pharmacokinetics (PK), safety and efficacy for six months. Blood for paromomycin and gentamicin PK parameters was collected from adult subjects after the first days' and last days' drug application. The primary efficacy endpoint was cure of a parasitologically confirmed index lesion, defined as at least 50% reepithelialization of the lesion by Day 63 and 100% reepithelialization by Day 100 with no relapse. The index lesion cure rate after 6 months follow-up was 13/15 (87%) for WR 279,396 and was 9/15 (60%) for Paromomycin Alone ($p = 0.099$). When all treated lesions were evaluated for cure, the final cure rate for WR 279,398-treated patients was the same, but the final cure rate for Paromomycin Alone-treated patients was lower at 8/15 (53.3%; $p = 0.046$). Both creams were well tolerated with mild application site reactions including erythema (20%), edema (13.3%), and pain (6.7%) being the most frequent adverse event in the WR 279,396 group, which were slightly higher in the Paromomycin Alone group. PK data showed that there is limited paromomycin and gentamicin systemic absorption thus avoiding drug accumulation and toxicity. The increased final cure rate in the WR 279,396 group in this small Phase 2 study suggests that the combination product may provide greater clinical benefit than paromomycin monotherapy against L. panamensis CL. The excellent tolerability and low systemic drug exposure suggests that WR 279,396 may offer an alternative to more toxic systemic therapies for CL.

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A PROSPECTIVE REFERRAL HOSPITAL STUDY OF SEVERE *PLASMODIUM KNOWLESI* MALARIA IN SABAH, MALAYSIA: HIGH INCIDENCE BUT NO MORTALITY WITH EARLY REFERRAL AND ARTESUNATE THERAPY

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The simian parasite *Plasmodium knowlesi* is a common cause of severe malaria in Malaysian Borneo, and high case-fatality rates have been reported with chloroquine and/or quinine treatment. We compared risk, spectrum and outcome of severe disease from *P.knowlesi, P.falciparum* and *P.vivax* following the introduction of early referral and intravenous artesunate for all severe malaria. From September 2010-October 2011 we prospectively recorded clinical and laboratory features of non-pregnant patients ≥12 years-old admitted to Queen Elizabeth Hospital (QEH), Sabah, with PCR-confirmed malaria monoinfection. Standardised referral (4+ parasite-density and/or any severity-criterion) and intravenous artesunate was instituted at district hospitals. Severe malaria (modified-WHO 2010 criteria) occurred in 38/130 (29%) patients with *P. knowlesi*, 15/122 (12%) with *P. falciparum* and 7/43 (16%) with *P. vivax*. Severity criteria in knowlesi malaria included hyperparasitemia (>100,000 parasites/μL, N=18), respiratory distress (N=14), jaundice (N=20), acute renal failure (N=9), hypotension (N=13), metabolic acidosis (N=4), anaemia (N=2) and abnormal bleeding (N=2). Severe knowlesi malaria occurred in 27/57 (47%) patients ≥50 years old compared to 11/73 (15%) <50 years. However using logistic regression, only parasite density independently predicted severe malaria, excluding hyperparasitemia as a sole severity-criterion (OR [log-increase in density count] 2.01, $p<0.0001$). Nearly all (92%) patients with knowlesi malaria received artemisinin therapy; 36/38 (95%) and 39/92 (42%) patients with severe and non-severe disease respectively received ≥1 dose of intravenous artesunate. Median parasite clearance-time was 2 days and no deaths occurred from any species. *P.knowlesi* is the commonest cause of severe malaria at QEH. Parasite density and schizontemia >10% were the only independent risk-factors for severity. Early treatment with artesunate was highly effective and associated with zero mortality.

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EFFECTIVENESS OF INTERMITTENT PREVENTIVE TREATMENT WITH SULFADOXINE-PYRIMETHAMINE IN PREGNANT WOMEN IN WESTERN KENYA: RESULTS OF AN OBSERVATIONAL STUDY

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Intermittent preventive treatment with sulfadoxine pyrimethamine (IPTp) remains a key strategy for malaria prevention in pregnant women living in malaria endemic regions. However, increasing SP resistance threatens IPTp effectiveness. We assessed the effectiveness of IPTp in an area of western Kenya where *Plasmodium falciparum* resistance to SP is high. From August 2008 to June 2009, women presenting to deliver at two district hospitals were enrolled in a cross-sectional survey. We collected information on obstetric history, use of IPTp, insecticide treated nets, and antimalarial treatment during pregnancy. At delivery, we measured the prevalence of maternal anemia (Hb< 8g/dL), peripheral parasitemia, placental parasitemia (impression smear) and low birth weight (LBW) by number of IPTp doses received by self-report or as recorded in the antenatal card. Overall 977 HIV-negative women were enrolled. Of these 637 (65%) were gravidae 1 or 2 and 340 (35%) were gravidae 3+. Among gravidae 1 or 2, anemia prevalence in women who received no IPTp was 14%, 1 dose: 11%, 2 doses: 7% and 3+ doses: 2% ($p<0.01$). Peripheral parasitemia was 19% for no IPTp, 12% for 1 and 2 doses and 7% for 3+ doses ($p=0.07$). Placental parasitemia was 22% for no IPTp, 12% for 1 dose, 13% for 2 doses and 8% for 3+ doses ($p=0.04$). Among gravidae 1/2, we found no reduction in LBW by IPTp doses administered ($p=0.73$). Among multigravidae, significant trends by number of SP doses received were not observed for anemia, or peripheral or placental parasitemia but were associated with reduced LBW ($p=0.02$). Among gravidae 1 or 2, but not multigravidae, having received more doses of IPTp was significantly associated with lower prevalence of maternal anemia, peripheral parasitemia and placental parasitemia. In multigravidae, IPTp resulted in reduced prevalence of LBW. During this time period, IPTp remained beneficial in this area of western Kenya, despite high SP resistance.

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INVASIVE *STAPHYLOCOCCUS AUREUS* INFECTIONS IN CHILDREN IN THE TROPICAL TOP END OF AUSTRALIA: CLINICAL AND MICROBIOLOGICAL EPIDEMIOLOGY

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Previous studies report very high incidence of *Staphylococcus aureus* bacteraemia in adult Aboriginal populations in tropical northern Australia. There are few studies describing incidence or outcomes in children. We aimed to describe the clinical and microbiological epidemiology of invasive *S. aureus* infections in children. We conducted a retrospective review for all cases of bacteraemia and sterile site infection, for children under 15 years, in the Top End of Australia over a four year period (2007–2010). Forty-four cases (9 neonatal, 35 paediatric) were identified. The annual incidence of invasive *S. aureus* was 27.9 cases per 100,000 population and was significantly higher in the Aboriginal population (incidence rate ratio [IRR] compared to non-Aboriginal population: 5.3 [95%CI 2.5-12.6]). Among non-neonatal cases, the annual incidence was 22.2 per 100,000 population (Aboriginal 46.6, non-Aboriginal 4.4, IRR: 10.6 [95%CI 3.8-41.4]). There were significant differences between the neonatal and paediatric groups. Neonatal cases were all born prematurely, typically with significant comorbidities and episodes were often intravascular catheter related and nosocomially acquired. There was one death. Of the 35 paediatric (non-neonatal) cases, 17% had pre-existing comorbidities, 14% were malnourished and 11% were nosocomial. Major foci of infection were bone and joint (57%) and pleuropulmonary (17%); endocarditis was uncommon (6%). The median length of stay was 23 days (mean 27, SD 16.6, range 2-68). 14% were readmitted within 1 year for related reasons. There were no deaths. There were 9 cases (26%) due to community-associated MRSA strains. Molecular genotyping results will be presented. There was no difference in severity or outcome between infections due to MRSA and MSSA. In conclusion, the annual incidence of invasive *S. aureus* infection in this study is the highest described in any paediatric population. Almost 1 in 2000 Aboriginal children develop invasive disease each year. Late onset sepsis in premature infants is the main neonatal cause. Most paediatric cases were community acquired and severe, yet rates of mortality, endocarditis and readmission were low.

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TRYPANOSOMA CRUZI SURVIVAL FOLLOWING STORAGE: IMPLICATIONS FOR TISSUE TRANSPLANTATION-DERIVED TRANSMISSION

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Trypanosoma cruzi, the causative agent of human Chagas disease, is typically transmitted via the feces of a reduviid bug vector following a blood meal. Infection can also occur through infected blood and tissue products. An estimated 300,000 individuals in the United States are infected with *T. cruzi* and six cases of transmission through organ donation have been reported. The potential for transmission by tissue transplantation is considered herein. Tissue recovered for transplantation, in contrast to solid organ transplants, undergoes a range of storage and processing procedures depending on the tissue type. At the outset, tissues do not have to be recovered from a beating-heart or live donor, but instead may be removed from the donor up to 24 hours after asystole. Once removed, tissues are often stored and eventually undergo some degree of processing, which can include lyophilization, irradiation, and various sterilization techniques. As an example, heart valves are cryopreserved and undergo minimal processing, whereas other tissue may be stored as is at -80 C for as long as five years. The ability of infected tissue to transmit *T. cruzi* is likely related to the ability of *T. cruzi* to survive these processing and storage conditions. We examined the viability of *T. cruzi* parasites after room temperature and cold storage conditions. *T. cruzi* trypomastigotes were unaffected by 24 hours at room temperature, both in cell cultures and when spiked into whole blood to mimic decomposition following asystole. Parasite-infected cells stored up to 5 days at 4 C proved 100% viable after re-culture, whereas only 2 of 8 cultures stored 28 days at 4 C became culture-positive. A significant decrease in parasite viability was observed in samples stored up to 120 days at -80 C in the absence of cryopreservation, yet some live, infective parasites were recovered. These data demonstrate the heartiness of *T. cruzi* following cold storage. Studies are underway to examine the effects of more rigorous tissue processing procedures on *T. cruzi* viability.

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A SYSTEMATIC REVIEW AND META-ANALYSIS OF MALARIA AND SEXUALLY TRANSMITTED AND REPRODUCTIVE TRACT INFECTIONS IN PREGNANCY IN SUB-SAHARAN AFRICA: OPPORTUNITIES FOR ANTENATAL INTERVENTION

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Malaria and sexually transmitted infections/reproductive tract infections (STIs/RTIs) in pregnancy are direct and indirect causes of stillbirth, prematurity, low birth weight, and maternal and neonatal morbidity and mortality. Novel use of diagnostic tools and/or drugs may improve birth outcomes with the impact depending on the prevalence of malaria and STI/RTI. PubMed, MEDLINE, EMBASE, the World Health Organization International Clinical Trials Registry, and reference lists were searched for studies reporting malaria, syphilis, *Neisseria gonorrhoeae, Chlamydia trachomatis, Trichomonas vaginalis*, or bacterial vaginosis among pregnant women attending antenatal care facilities in sub-Saharan Africa. Included studies were conducted in 1990-2011 with open enrollment. Studies from South Africa, where malaria is no longer endemic, were excluded. Point prevalence estimates were corrected for diagnostic precision. A random-effects model meta-analysis was then applied to produce pooled prevalence estimates. A total of 171 studies met inclusion criteria, providing 307 point prevalence estimates for malaria or STIs/RTIs. The pooled prevalence estimates (95% confidence intervals; n=positive diagnoses) among studies in 1990-2011 in East and Southern Africa were as follows: syphilis 4.5% (3.9,5.1; n=8,346), *N. gonorrhoeae* 3.7% (2.8, 4.6; n=626), *C. trachomatis* 6.9% (5.1, 8.6; n=350), *T. vaginalis* 29.1% (20.9, 37.2; n=5,502), bacterial vaginosis 50.8% (43.3, 58.4; n=4,280), peripheral malaria 32.0% (25.9, 38.0; n=11,688) and placental malaria 25.8% (19.7, 31.9; n=1,388). West and Central Africa prevalence estimates were as follows: syphilis 3.5% (1.8, 5.2; n=851), N. gonorrhoeae 2.7% (1.7, 3.7; n=73), C. trachomatis 6.1% (4.0, 8.3; n=357), T. vaginalis 17.8% (12.4, 23.1; n=822), bacterial vaginosis 37.6% (18.0, 57.2; n=1,208), peripheral malaria 38.2% (32.3, 44.1; n=12,242) and placental malaria 39.9% (34.2, 45.7; n=4,658). In conclusion, the dual prevalence of malaria and STIs/RTIs in pregnancy among women who attend antenatal care facilities in sub-Saharan Africa is considerable, with the combined prevalence of curable STIs/RTIs being equal to, if not greater than, malaria.

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ANSWERING THE MAIL: USING A CASE-BASED MODEL TO TEACH TELECONSULTATION SKILLS TO INFECTIOUS DISEASE FELLOWS

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The United States Military has offered a vast array of teleconsultative services to assist health care providers (HCP) deployed in remote areas of the world via e-mail. The Infectious Diseases Teleconsult system (IDTS) has been an effective tool in optimizing care for Service Members overseas. Despite the continuing development of telemedicine in both civilian and military communities, we are unaware of any standardized education given to HCP prior to participating in this method of consultation. Giving medical recommendations via a teleconsult system requires unique skills not used in the standard clinical setting. Consultants provide advice without questioning or examining the patient and must understand system constraints unique to the patient's location (i.e. isolated mountain top in Afghanistan or remote village in West Africa). Cases referred to the IDTS often involve diseases unique to tropical climates and the developing world that inexperienced providers may not recognize. To address this gap in medical education, the Infectious Diseases (ID) fellowship program at Walter Reed National Military Medical Center implemented a training program in 2011 that utilized a series of simulated patients based upon real teleconsults from the IDTS system. Ten cases were chosen to highlight classic infectious diseases that have been common consults such as cutaneous Leishmanisis, Q fever and malaria. The simulated cases are administered via e-mail to an ID fellow who then has one hour to appropriately answer the teleconsult. The fellow is then given feedback on his/her ability to generate an accurate assessment and plan for the simulated case and the practicality of their advice considering the patient care setting. An overview of the case-based model, selected examples, and evaluation criteria will be reviewed. This educational process is an effective way to prepare ID fellows for the real-world experience of offering advice via a teleconsult service and has improved their knowledge base of infectious diseases in the developing world.

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MATERNAL HIV EXPOSURE OR MOTHER TO CHILD TRANSMISSION OF HIV AND RISK OF TUBERCULOSIS IN INFANTS

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Infants born to HIV infected mothers who test HIV negative (seroexposed) and those that become HIV infected (seroreactive) are at an increased risk of progression from latent tuberculosis (TB) infection to disease. However, TB diagnosis in this group is challenging due to low utility of scoring systems and low tuberculin skin test (TST) sensitivity. We sought to document the incidence rates of TB disease among seroexposed and seroreactive babies, among an infant cohort under follow up to determine TB incidence. Infants aged 0-6 weeks were recruited from Western Kenya. HIV DNA PCR testing was offered at six weeks to all infants. Through follow up and sick visits, TB suspects were identified based on signs and symptoms and investigated for TB through gastric aspirates, sputum induction, chest radiographs, HIV tests and TST. Definite cases were were culture confirmed and probable cases had Keith Edward(KE) Scores of ≥7 and/or suggestive chest radiographs. Of 2900 infants enrolled, 435 (15%) were seroexposed, including 75 (2.6%) seroreactive. Of 923 (32%) TB suspects, 174 (19%) out of 923 TB suspects were seroexposed, including 43(25%) seroreactive. The mean TST induration among sero exposed and non exposed suspects was 5mm(SD=6) and 4mm (SD=5) $p<0.01$ respectively. Additionally, mean KE score was 2.3 (SD2.4) and 1.6 (SD2.0) $p<0.01$ for seroexposed and non-exposed respectively. There were 48 TB cases, (incidence rate 1.44 per 100 person years) [95% CI (1.09, 1.91)], sixteen of these (33%) were among seroexposed infants RR 2.1 [95% CI(1.1,4.0)]. Out of ten definite TB cases, five (50%) were seroexposed ($p=0.05$). HIV exposure and infection was associated with increased risk of being diagnosed with tuberculosis. HIV infected mothers have increased risk of developing TB and exposing their babies to TB infection. Rigorous antenatal screening for tuberculosis in HIV infected mothers is recommended to protect their infants from early latent infection and progression to TB disease.

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INCREASED RATES OF RESPIRATORY AND DIARRHEAL ILLNESSES AMONG HIV-NEGATIVE INDIVIDUALS ≥5 LIVING WITH HIV-INFECTED INDIVIDUALS IN AN URBAN SLUM (KIBERA) OF NAIROBI, KENYA

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For some acute illnesses in HIV-infected patients, prolonged pathogen shedding, symptom duration, and other socioeconomic factors potentially contribute to increased incidence. Using data from a prospective, population-based infectious disease surveillance system of acute illnesses and a cross-sectional HIV serosurvey conducted in the urban slum of Kibera, Kenya, we calculated incidence of influenza-like illness (ILI), diarrhea, and non-specific febrile illness among 2,105 HIV-negative household contacts of HIV-infected participants and 13,747 participants living within exclusively HIV-negative households during 2008. Of the 4,285 household in which a test was performed, 83.8% had only HIV-negative tests, 13.5% had 1 HIV-positive test, and 2.7% had more than 1 HIV-positive test; untested adults were not included in the analysis. We stratified household contacts by number of HIV-infected individuals in households and modeled it as a continuous variable to determine a dose-dependent relationship. For children and adults ≥5 years old, incidence was significantly increased for ILI (incident rate ratio [IRR], 1.47; 95% confidence interval [CI], 1.07:1.99; $p<0.05$), and diarrhea (IRR, 1.41; CI, 1.11:1.77; $p<0.05$) in HIV-negative household contacts of HIV-infected residents when compared with rates in HIV-negative persons living in households without an HIV-infected resident. The risk for illness among HIV-negative persons was directly proportional to the number of HIV-infected people living in the home for ILI (IRR, 1.40; CI, 1.03:1.88; $p<0.05$), diarrhea (IRR, 1.36; CI, 1.11:1.67; $p<0.01$), and non-specific febrile illness (IRR, 1.15; CI, 1.02:1.29; $p<0.05$). There was no evidence for a similar pattern of increased incidence in a parallel analysis among children <5 years old. Since treatment and support for HIV-infected individuals reduces their incidence of infections, future interventions ensuring that HIV-infected persons are receiving appropriate care may help to reduce infections for all household members.

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RUSH TO JUDGMENT: SOURCES OF CONFOUNDING IN STI-HIV PREVENTION TRIALS

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Substantial evidence indicates that sexually transmitted infections (STIs) promote HIV transmission and acquisition by producing genital ulcers, inflammation, and viral shedding. Ten randomized controlled trials in sub-Saharan Africa (SSA) examined effects of STI control on HIV incidence. One produced statistically significant results. Consequently, support for STI treatment for HIV prevention has faded. We conducted an intensive review of methods and outcomes of the 10 STI-control trials in SSA and subsequent analyses. All 10 trials reveal potentially serious confounding from multiple untreated genital morbidities. Some trials studied the impact of treating bacterial STIs on HIV incidence; others studied treatment of viral STIs. None studied both. None examined treatment of genital morbidity from other causes that could enhance HIV transmission or acquisition. The trials excluded consideration of fungal STIs. None considered genital ulceration and inflammation from non-sexually transmitted pathogens, such as Schistosomiasis hematobium (highly prevalent in SSA), and from ulcers caused by abrasions infected with streptococci or staphylococci, also common. Treating one type of genital morbidity may have little effect on HIV incidence when there is untreated genital morbidity from multiple sources. Furthermore, 8 trials reported the same or lower levels of risky sexual behavior in the control arm as in the treatment arm or reported the same or larger reductions in risky behaviors among controls. (Two trials did not report on sexual behavior.) That could have resulted from the trials' successful interventions in the control arm or from spontaneous reductions in risky behavior prompted by the trial. Confounding by genital morbidity of multiple etiologies, behavioral change, and other factors in the 9 trials lacking statistically significant results renders those trials unable to inform HIV-prevention policy. Given abundant evidence that STIs promote HIV spread, STI treatment should be considered an important method for reducing HIV incidence in SSA and elsewhere.

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HIV STIGMA AS A BARRIER TO RECEIVING HIV CARE AT A GENERAL HOSPITAL IN LIMA, PERU: A CASE-CONTROL STUDY

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Poor retention in care may increase the risk of morbidity, mortality, and community HIV transmission. The role of HIV stigma in poor retention has not been well studied. The objective of this case-control study was to evaluate the association between HIV stigma and retention in care among HIV patients in Lima, Peru. We evaluated HIV-positive patients who were diagnosed and/or initiated care at a general hospital between 2005-2010, with inclusion based on status of care by March 31, 2011. Those retained in care (n=150) had ≥ 2 documented medical care visits per year and were approached and interviewed privately in clinic during their appointment. Those not retained in care (n=55) had no documented visits for ≥ 1 year and home visits were used to locate them and conduct interviews. The Berger HIV stigma scale was used to quantify the 4 domains of stigma: enacted stigma (ES), disclosure concerns (DC), negative self-image (NSI), and concern with public attitudes (CPA). Each domain had 5 items with higher scores indicating higher stigma (score range 0-15). Multivariable logistic regression was used to calculate adjusted odds ratios (OR) and 95% confidence intervals (CI) for being out of care. Stigma was modeled as a continuous variable and linearity assumptions were assessed. Mean stigma scores were low for ES (6.1) and NSI (5.3) but high for DC (9.6) and CPA (9.0). ES and NSI had U-shaped associations with retention (odds of not being retained increased then decreased at higher stigma levels). DC and CPA showed linear associations. Patients who agreed to all items (score of 10) were more likely to not be retained than patients who disagreed to all items (score of 5) for ES (OR=2.36; 95% CI: 0.98, 5.67), DC (OR=2.72; 95% CI: 1.11, 6.67), NSI (OR=1.82; 95% CI: 0.50, 6.60), and CPA (OR=3.30; 95% CI: 1.37, 7.92). This study suggests that all aspects of HIV stigma, particularly concern with public attitudes, play a role in being out of care.

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DO PEOPLE LIVING WITH HUMAN IMMUNODEFICIENCY VIRUS (PLHIVS) HAVE MORE RISKY SEXUAL BEHAVIORS THAN UNINFECTED PEOPLE IN BURKINA FASO? A CASE-CONTROL STUDY

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Antiretroviral therapy has reduced mortality and morbidity due to HIV/AIDS. Consequently, it is widely believed that PLHIV could keep new infections occurrence by their more risky sexual behaviors. This study aimed to compare PLHIVs' sexual behaviors with those who are infected, and to determine factors that affect their condom use. We conducted a case-ontrol study involving all the PLHIVs and not infected people of the second round in Burkina Faso's Treatment Accelerating Program assessment, from July to December 2009. This assessment involved PLHIVs (with their households) and households without PLHIV. Chi-square test was used to compare proportions with $p<0.05$ as statistically significant. Logistic regression was employed to determine associated factors of condom use. Two hundred ninety one PLHIVs and as many controls were interviewed in the 590 households. Seventy four percent of PLHIVs versus 58.1% of controls were women ($p< 0.001$). Among men, 78.7% of PLHIV and 84% of controls had sex in the last twelve months ($p>0.05$). Among women, we had 48.1% versus 56.4% of PLHIV ($p>0.05$). three percent of PLHIV versus 13% of controls were not aware of their last sexual partner's HIV status ($p< O.05$). Among PLHIV, 27.1% versus 67% of the controls had not used condom during their last sexual intercourse ($p< 0.001$). Both men's and women's group had presented the same difference ($p<0.001$). ART status was not associated with condom use (OR=1.2, 95%CI=0.6-2.5). But, voluntary testing (OR=3.4, 95%CI=1.1-10.7) and knowing that the partner has had sex with another person during the last twelve months (OR =5.2, 95%CI=1.6-16.7) were associated with PLHIV's condoms use. PLHIVs use condom more than uninfected and in general, they don´t have more risky sexual behaviors. Besides, condom use is not significantly affected by ART, so their availability doesn't increase risky behaviors and therefore must be improved. This argument could be considered to strengthen public awareness campaigns against stigma and discrimination against PLHIVs in Burkina Faso.

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RELATIVE EXPRESSION OF CCR5 AND CXCR4 BY CD14+ MONOCYTES AND CD4+ T CELLS IN HIV-1-EXPOSED AND -INFECTED CHILDREN WITH *PLASMODIUM FALCIPARUM* MALARIA

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We and others have previously shown that *Plasmodium falciparum*-derived hemozoin (*Pf*Hz) promotes dysregulation of CCR5 and CXCR4, and their cognate ligands in monocytes and CD4+ T cells, resulting in increased HIV-1 replication. To further explore these molecular interactions, we examined the relative expression of CCR5 and CXCR4 on CD14+ monocytes and CD4+ T cells through flow cytometric analyses on cells collected from children (age, 2.6-33.6 mos; n=67) from western Kenya categorized into the following groups: 1) *P. falciparum* negative and HIV-1 negative [mal(-)/HIV-1(-), n=11]; 2) *P. falciparum* positive and HIV-1 negative [mal(+)/HIV-1(-), n=33]; 3) *P. falciparum* positive and HIV-1 exposed [mal(+)/HIV-1(exp), n=20]; and 4) *P. falciparum* positive and HIV-1 positive [mal(+)/HIV-1(+), n=3]. Proportions of CD14+CXCR4+ cells were elevated in mal(+)/HIV-1(+) children compared to the mal(-)/HIV-1(-) group (P=0.048). However, proportions of CD14+CXCR4+ cells were reduced in the mal(+)/HIV-1(+) children relative to mal(+)/HIV-1(-) (P=0.039) and mal(+)/HIV-1(exp) (P=0.077) groups. In addition, expression of CD3+CXCR4+ and CD3+CD4+CCR5+ cell subsets were lower in the mal(+)/HIV-1(+) group compared to mal(+)/HIV-1(exp) children (P<0.05 for both). Further analyses in the combined population of malaria-infected children revealed that CD3+CXCR4+ cells were inversely correlated with the percentage of monocytes containing *Pf*Hz (ρ=-0.883, P=0.020). Taken together, the preliminary results presented here suggest that CXCR4 and CCR5 are dysregulated in children co-infected with malaria and HIV-1, and that altered expression may be driven, at least in part, through acquisition of *Pf*Hz by monocytes.

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AN EVALUATION OF TB AND HIV PROGRAM INTEGRATION AT PRESIDENT'S EMERGENCY PLAN FOR AIDS RELIEF (PEPFAR)-SUPPORTED MILITARY HOSPITALS IN NIGERIA

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Tuberculosis (TB) presents an important health problem and comorbidity among patients seen at sites supported by the President's Emergency Plan for AIDS Relief (PEPFAR) in Nigeria. The objective of this evaluation was to use the TB cohort review process indicators to assess TB and HIV program integration at PEPFAR sites within the Nigerian Ministry of Defense (NMOD). The cohort review methods used were modified from those described previously by the Centers for Disease Control and Prevention. The sites were all affiliated with the NMOD and included clinics in Abuja, Lagos (2 sites), Makurdi, and Kaduna. At each site, the study team reviewed each TB case in detail with the assistance of the TB case manager, verifying key clinical outcomes. TB case managers were interviewed for qualitative information about the processes involved in TB care at each facility. 175 cases were reviewed at the 5 PEPFAR-supported sites. The sites evaluated were found to have substantial variability in outcomes. Treatment completion seen in this study (72%) was lower than that reported nationally (83%) and was lower than the WHO 2015 goal of (85%). Of particular concern, HIV patients were less likely to complete therapy (58%) than HIV negative patients (85%). The lack of contact investigation and TB case finding supports the low case detection rate reported nationally (20%). Similarly, the lack of LTBI treatment is consistent with the low proportion of treatment among eligible HIV patients (<5%) seen nationally. This is the first report of the cohort review methodology being applied to assess TB program integration within a PEPFAR population. Assessments of TB program integration through the cohort review can benefit the PEPFAR program by increasing staff awareness and accountability for patient outcomes, improving case management and investigation of contacts, improving monitoring and evaluation practices, and revealing program strengths and weaknesses. This evaluation has provided data to fill some of the gaps related to integration of TB control in a PEPFAR population.

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THE JOINT EFFECTS OF EFFICACY AND COMPLIANCE: A STUDY OF HOUSEHOLD WATER TREATMENT EFFECTIVENESS

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The effectiveness of interventions to control infectious disease is related to the intrinsic efficacy of the interventions in removing pathogens, and how people comply with the interventions. However, little is known about the quantitative relationship between compliance and effectiveness, which is particularly important for household water treatment (HWT). Although many HWT methods are highly efficacious at inactivating pathogens, their effectiveness within actual communities is decreased by imperfect compliance. To assess the effectiveness of HWT on childhood diarrhea incidence via drinking water for three pathogen types (bacterial, viral, and protozoan), we developed a quantitative microbial risk assessment (QMRA) model. We examined the relationship between log10 removal values (LRVs) and compliance with HWT for scenarios varying by: baseline incidence of diarrhea; etiologic fraction of diarrhea by pathogen type; pattern of compliance; and size of randomly scheduled contamination spikes in source water. The benefits of increasing LRVs are strongly linked to compliance. For perfect compliance, diarrheal incidence decreases as LRVs increase. However, when compliance is incomplete in the scenarios we considered, there are diminishing returns from increasing LRVs at the community level. Higher LRVs are more beneficial if: contamination spikes are large; contamination levels are high in general; or the pattern of compliance includes some people who comply perfectly. The effectiveness of an HWT intervention at the community level may be limited by low compliance, such that the benefits of high LRVs are not realized. Therefore, patterns of compliance with HWT should be measured during HWT field studies and HWT dissemination programs. Studies of pathogen concentrations in a variety of developing country source waters should also be conducted. Guidelines are needed for measuring and promoting compliance with HWT, in addition to the recently published WHO HWT efficacy recommendations.

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SPATIOTEMPORAL DYNAMICS OF CHOLERA EPIDEMICS IN THE FAR NORTH REGION, CAMEROON

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In the year 2010, the Far North Region of Cameroon experienced its most severe cholera outbreaks in the last four decades with about 9,400 reported cases and 600 deaths. This study describes the spatiotemporal dynamics of cholera epidemics from 1996 to 2010 using epidemiological data from this region, including reported cholera cases from 28 health districts, and environmental parameters such as temperature, relative humidity, rainfall and access to clean water. The data were entered into a geographical information system for further analysis. Regression analysis methods were used to analyze the data. The spatiotemporal patterns of the incidence rate were analyzed and associated with environmental factors to explore the determinant factors of the dynamics of cholera epidemics. The results revealed that there were three major epidemic periods and specific hotspots during the last 15 years. The annual dynamics showed a seasonal pattern coinciding with the wet seasons and significant differences in both incidence and timing by health districts. The spatial pattern revealed higher incidence rate in health districts in proximity with water bodies and in periurban areas. The study also revealed a connection with outbreaks in the neighboring countries of Chad and Nigeria. This study presents information related to the epidemiology and spatiotemporal pattern of cholera epidemics that can be used to help public health services plan prevention and control strategies against the spread of this disease.

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THE RELATIONSHIP BETWEEN DISTANCE TO HOUSEHOLD WATER SOURCE AND MODERATE-TO-SEVERE DIARRHEA IN YOUNG CHILDREN IN THE GLOBAL ENTERICS MULTI-CENTER STUDY (GEMS), KENYA, 2009-2011

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Water sources for rural households in the developing world are often located away from the home. Fetching water can be a substantial burden that can negatively affect household water quantity and quality, thereby increasing diarrheal disease risk. We visited households of 127 randomly selected pairs of case and age-, gender-, and village-matched controls enrolled in the GEMS study of moderate-to-severe diarrhea (MSD) in Kenya. We asked households to guide field teams from their home to their water source, and this path was captured as spatial data using GPS units. If no guide was available, we used GPS coordinates of the home and the water source to estimate distances. We compared GPS-sourced data to self-reported data in GEMS about round trip times spent to collect water, and evaluated each type of measurement as a predictor of MSD using conditional logistic regression. The paths recorded were a median of 1.18 (range 1.00 - 2.49) times the length of the straight line distances between their start and endpoints. Self-reported collection times were significantly correlated with the log-transformed distance measurements via GPS (Spearman correlation coefficient =0.80, p <0.01). The median recorded distance to water source was 196m (range: 1 - 1775m); 89 (35%) households were within 50m, all of which also reported the source to be in the household area. Collection times of 30 - 59 minutes were reported by households of 24 cases (median distance 561m, range 100 - 1775m) and 8 controls (median 562m, range 197 - 981m), and the odds of MSD were significantly higher vs. those with no travel (p <0.01). Collection times longer than 1 hour were reported for 12 cases (median distance 744m, range 148 - 1466m) and 3 controls (median distance 530m, range 460 - 1048m) and were significantly associated with MSD (p=0.02). These data suggest that distances traveled by households in rural Kenya to fetch water varied widely, that self-reported water collection times are correlated with measured distances, and that these may be useful in multivariate analyses of risk factors for MSD.

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RELATIONSHIP BETWEEN USE OF WATER FROM COMMUNITY-SCALE WATER TREATMENT REFILL KIOSKS AND CHILDHOOD DIARRHEA IN JAKARTA

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In developing countries, safe piped drinking water is generally unavailable, and bottled water is unaffordable for most people. Purchasing drinking water from community-scale decentralized water treatment and refill kiosks (referred to as isi ulang depots in Indonesia) is becoming a common alternative. This study investigates the association between diarrhea risk and water kiosks. We monitored daily diarrhea status and water source for 1,000 children aged 1--4 years in Jakarta, Indonesia, for up to 5 months. Among children in an urban slum, rate of diarrhea per 1,000 child-days varied significantly by primary water source: 8.13 for tap water, 3.60 for bottled water, and 3.97 for water kiosks. In multivariable Poisson regression analysis, diarrhea risk remained significantly lower among water kiosk users (adjusted rate ratio [RR] = 0.49, 95% confidence interval [CI] = 0.29--0.85) and bottled water users (adjusted RR = 0.44, 95% CI = 0.21--0.94), compared with tap water users. Purchasing water from low-cost water kiosks is associated with a reduction in diarrhea risk similar to that found for bottled water.

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FOOD PREPARATION PROCESSES AND HYGIENE PRACTICES IN RURAL BANGLADESH: OPPORTUNITIES TO IMPROVE HANDWASHING INTERVENTIONS

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In rural Bangladesh handwashing with soap during food preparation is associated with reduced child diarrhea, but we have limited data characterizing food preparation and related handwashing to inform behavior change interventions. This study explored the steps of food preparation, related handwashing opportunities, community perceptions and current practices regarding handwashing during food preparation. In 3 rural Bangladeshi villages, we conducted 12 in-depth interviews, 12 unstructured observations and 12 video observations with caregivers, and 3 focus group discussions with household heads on handwashing related to food preparation. Eating, preparing and serving food with bare

hands was common in the study communities. Varieties of mashed foods, salads and mixed dried foods that involved direct hand contact, which are not further cooked, were popular in rural Bangladesh. Mashed foods are prepared by boiling vegetables or dried fish, then peeling, mashing and mixing with raw ingredients using bare hands. Salad preparation involved cutting raw vegetables and mixing them by hand. For mixed dried foods puffed rice and dried snacks are hand-mixed with raw ingredients. Participants perceived that handwashing with soap was only necessary if hands were covered with visible dirt. Most respondents reported that they wash their hands with water during food preparation, but we observed that out of 54 opportunities to wash hands, participants washed hands with soap 2 times, with water alone 9 times, rinsed hands or hands came into contact with water 26 times, and did not wash hands 17 times. Food preparation was often interrupted by other tasks that could contaminate the preparer's hands, after which they continued food preparation without washing hands. Participants cited that absence of soap in appropriate place is a potential barrier to wash hands with soap. Caregivers do not usually wash their hands with soap during food preparation in rural Bangladesh. Food preparation is a complex, multi-step, often interrupted process where villagers do not recognize moments of high risk of environmental contamination as a time to wash hands with soap. Identifying the highest risk food preparation steps and prioritizing those when handwashing with soap is important will help focus handwashing interventions. Bringing soap and water together in the food preparation area may make it easier to wash hands with soap during such high risk moments.

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INACTIVATION OF HELMINTH IN A SOLAR CONCENTRATOR

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More than 1 billion people worldwide are infected with helminths. Typical pit latrines and composting conditions do not inactivate helminths in fecal matter effectively. By concentrating solar energy and reaching pathogen inactivation temperatures (50°C and higher), a solar concentrator, with projected capital costs of $0.30 per person per year, has the potential to inactivate helminths in fecal matter. The goal of this work was to evaluate the efficacy of a solar concentrator in inactivating helminth in fecal matter and meet World Health Organization (WHO) guidelines for safe disposal and reuse of fecal matter. Inactivation was assessed by evaluating the viability of *Toxocara canis* eggs. *T. canis* is a helminth in the same taxonomic order as *Ascaris lumbricoides*, which is a WHO indicator for safe fecal disposal and reuse. Three trials were conducted from December 2011 through February 2012 in Santiago, Chile. The first two trials evaluated *T. canis* viability daily. To calculate the inactivation rate for the solar concentrator unit, the third trial evaluated *T. canis* viability hourly. In each trial, *T. canis* eggs were isolated from canine fecal matter, concentrated, placed in semi-permeable tea bags (1,500 eggs each) and inoculated into 40 liters of fresh canine fecal matter. *T. canis* eggs were inoculated into two control conditions: indoors in the dark and in a mimic pit latrine. At the end of each trial, eggs were incubated and classified as viable if they contained a motile larva. In all 3 trials, temperatures reached 60°C at the center and 70°C at the edges of the solar concentrator for at least 4 hours daily. During all three trials, after one day in the solar concentrator, the die-off of eggs was greater than 99%. In the third trial, the inactivation rate was 0.67 Log_{10} eggs/hr and there was 99% inactivation after 4 hours. These results suggest that a solar concentrating unit can be used to rapidly inactivate helminths in fecal matter, and therefore, fecal matter treated by a solar concentrator can be safely disposed and reused on edible crops.

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CONTINUED ENVIRONMENTAL FECAL CONTAMINATION FOLLOWING IMPLEMENTATION OF SANITATION HARDWARE

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Even with latrines available, child and animal feces are often found in and around households in rural Bangladesh. These feces may contain important gastrointestinal pathogens. We piloted child potties and a customized hoe like tool (sani scoop) to measure their impact on environmental fecal contamination. We distributed child potties in 104 rural households with children <3 years of age. All households in study household compounds were given sani scoops to encourage hygienic disposal of child feces in latrines and animal feces in designated pits. Local health promoters encouraged using scoops and potties in these households through nurture and disgust themed messages. Field workers administered a 3 month follow-up survey that included participant reported frequency of use, and field worker observation of the presence and condition of hardware and presence of animal and human feces around the household. We used in-depth interviews and focus group discussions with mothers from these communities to identify the barriers to using the hardware. Reported use of potties (67%) and sani scoops (89%) for child defecation events were high. Little difference in the presence of human feces was detected 3 months after receiving the intervention (19/96; 20%) compared to baseline (16/104; 16%), and to control households (22%). Similarly animal feces were found in 87/104 (84%) of intervention households at 3 months compared to 92/96 (96%) at baseline and 99% in control compounds. During in-depth interviews participants reported incomplete potty training, inconsistent potty use and delays in disposing feces because of their many other household tasks. Ubiquitous poultry and other domestic animals regularly produced fresh feces. The utility of cow dung as biofuel led to conflict over ownership of feces and appropriate handling. Perceptions of child and some animal feces as harmless limited household's motivation to dispose of feces. Though potties and sani scoops had high acceptability and self-reported use, most households maintained high levels of observable feces at follow-up. Although improving disposal of child feces is often mentioned as part of sanitary interventions, additional research is needed to develop practical strategies to reduce contamination in a child's household environment.

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LOOKING BEYOND KDR: THE EMERGENCE OF A NEW MUTATION, N1575Y, IN THE SODIUM CHANNEL OF *ANOPHELES GAMBIAE* AND ITS ROLE IN INSECTICIDE RESISTANCE

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Insecticide resistance is a major threat to malaria vector control. In *Anopheles gambiae*, resistance to pyrethroid and DDT insecticides is strongly associated with the mutations *L1014F* and *L1014S* within the para voltage-gated sodium channel (VGSC). Across much of West Africa, *1014F* frequency approaches fixation. Here, we document the emergence of a mutation, *N1575Y*, within the linker between domains III-IV of the VGSC. In data extending over 40 kbp of the VGSC *1575Y* occurs on only a single long-range haplotype, also bearing *1014F*. The *1014F-1575Y* haplotype was found in both M and S molecular forms of *An. gambiae* in West/Central African sample sites separated by up to 2,000 km. In Burkina Faso M form, *1575Y* allele frequency rose significantly from 0.053 to 0.172 between 2008 and 2010. Extended haplotype homozygosity

analysis of the wild-type *1575N* allele showed rapid decay of linkage disequilibrium (LD), in sharp contrast to the extended LD exhibited by *1575Y*. A haplotype with long-range LD and high/increasing frequency is a classical sign of strong positive selection acting on a recent mutant. *1575Y* occurs ubiquitously on a *1014F* haplotypic background, suggesting that the *N1575Y* mutation compensates for deleterious fitness effects of *1014F* and/or confers additional resistance to insecticides. Haplotypic tests of association suggest the latter: The *1014F-1575Y* haplotype confers a significant additive benefit above *1014F-1575N* for survival to DDT (M form P = 0.03) and permethrin (S form P = 0.003). DNA-based diagnostics are supplementing phenotypic bioassays as a proactive means of detecting resistance alleles at low frequency. The discovery of *N1575Y* at an early stage highlights the importance of continual monitoring for novel resistance mutations and its spread should be monitored closely.

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THE EVOLUTION OF RESISTANCE TO CARBAMATES AND ORGANOPHOSPHATE INSECTICIDES IN *ANOPHELES GAMBIAE*

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Determining the number of origins of insecticide resistance-associated mutations is important not only from an evolutionary perspective but also for modelling its spread, which is increasingly important for the implementation and monitoring of malaria vector control programmes. In the mosquito *Anopheles gambiae* s.s., the main African vector of malaria, organophosphate (OP) and carbamate resistance is strongly associated with a single amino-acid substitution (G119S) in the insecticide target site, acetylcholinesterase 1 (*Ace-1*). This mutation apparently incurs a high fitness cost in the absence of insecticidal pressure. Recently, a duplication of the *Ace-1* locus was observed in *An. gambiae*, which results in a wildtype and resistant-associated allele occurring on the same haplotype (*Ace-1^D*). It is proposed that the *Ace-1^D* haplotype is in effect a "fixed" heterozygote that may confer similar levels of resistance but with reduced fitness effects. We have screened for diversity in and around the *Ace-1* locus in mosquitoes from a large number of sites in West Africa. We present data showing a selective sweep centred on the *Ace-1* locus and extending for approximately 1MB in both centromeric and telomeric directions. Targeted sequencing of the *Ace-1* locus has revealed the first evidence of multiple origins of the resistance allele and a potentially more complex of pattern of duplication, with potentially important consequences for vector control involving OPs and carbamates.

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RESISTANCE TO PYRETHROID AND CARBAMATE THREATENS VECTOR CONTROL IN WEST OF TANZANIA

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Kagera region on the western side of Lake Victoria had the highest malaria burden in Tanzania according the 2007 Malaria Indicator Survey. To reduce malaria transmission an annual round of indoor residual spraying (IRS) has been conducted since 2007 initially with lambdacyhalothrin (pyrethroid) and more recently with bendiocarb (carbamate). A campaign of universal coverage of long lasting insecticidal nets (LLIN) was carried out in 2011. The emergence of resistance could threaten the future of these two interventions. As a component of a cluster randomized trial comparing the combination of LLIN and IRS versus LLIN alone the distribution of vectors and prevalence of insecticide resistance is being monitored. From April to December 2011, monthly *Anopheles* collection using CDC light traps was carried out across 40 villages in the area. Resistance monitoring was carried out on *An. gambiae* s.l. using WHO cylinder test. CDC bottle bioassays with synergists examined the involvement of metabolic resistance. Species identification and prevalence of knock down resistance (*kdr*) was confirmed using real time PCR TaqMan assay. A total of 5844 *Anopheles* mosquitoes were collected over seven months, of these 67% were collected in April, two months after spraying with pyrethroid. 81.8% were *An.gambiae* s.s. and 17.2% were *An. arabiensis*. East *kdr* mutation which is associated with pyrethroid and DDT resistance was present at high frequency in *An.gambiae* s.s. (97%) but only at 5% in *An.arabiensis.* Mortality in WHO resistance tests ranged from 0% to 38% for lambdacyhalothin, 12% to 40% for DDT, and 84% to 100% for bendiocarb. Result from the CDC bottle assay suggested the presence of elevated level of oxidases and esterases. East *kdr* mutation seems to have reached fixation in the *An.gambiae* s.s population. High phenotypic resistance to pyrethroid was observed. In contrast to neighbouring Kenya where *An.gambiae* s.s. nearly disappeared after vector control despite high *kdr* frequency, *An.gambiae* s.s. remains predominant in Kagera even with high coverage of pyrethroid IRS and LLINs.

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DISSECTING THE MOLECULAR BASIS OF PYRETHROID RESISTANCE IN FIELD POPULATIONS OF THE MAJOR MALARIA VECTOR *ANOPHELES FUNESTUS* IN SOUTHERN AFRICA

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Anopheles funestus is a major malaria vector in Southern Africa. It remains unclear whether the many reports of pyrethroid resistance in this region have the same underlying mechanisms spreading between countries through gene flow or if different mechanisms occurred independently. To elucidate these questions we dissected the molecular basis of pyrethroid resistance in three countries, Mozambique, Malawi and Zambia. Microarray analysis using an Agilent chip identified three main P450 genes associated with permethrin resistance (CYP6P9a, CYP6P9b and CYP6M7) but with significant differences in expression patterns between countries. Other genes potentially implicated involved a short chain dehydrogenase and other P450s such as CYP6AA4 and CYP9J14. The most upregulated gene in Mozambique is CYP6P9b with a fold-change (FC) >88, then CYP6P9a (FC~60) and CYP6M7 (FC~25). Interestingly in Malawi, CYP6P9a is the most upregulated gene (FC~69) then CYP6P9b (FC~30) and last CYP6M7 (FC~12) while in Zambia, CYP6M7 is the top upregulated gene (FC~37) before CYP6P9a (FC~15) and CYP6P9b (FC~11). The overall higher fold-change in Mozambique correlates with the higher level of resistance in this country. The upregulation of these genes was validated by qRT-PCR. Polymorphism analysis of these 3 genes and surrounding microsatellite markers detected selective sweep signatures for CYP6P9b and CYP6P9a but less for CYP6M7. Transgenic *In vivo* expression of CYP6P9a and CYP6P9b using the GAL4/UAS system indicated that both genes confer resistance in *Drosophila* to permethrin and deltamethrin. *In vitro* metabolism assays with recombinant proteins of both genes in *E coli* cells showed that CYP6P9a and CYP6P9b both metabolise Type I (permethrin) and Type II (deltamethrin and λ-cyhalothrin) pyrethroids but not Etofenprox or DDT. The cloning of the 6kb cDNA of the VGSC gene identified rare clones with potential kdr mutations which remain to be confirmed in field populations. Overall, these results suggest the presence of different resistance fronts in populations of *An. funestus* in Southern Africa.

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EVOLUTION OF INSECTICIDE RESISTANCE AND MALARIA POSITIVITY RATES IN *ANOPHELES GAMBIAE* AFTER INTRODUCTION LONG LASTING INSECTICIDE TREATED BED NETS IN DIELMO, SENEGAL

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Despite many efforts in basic and applied research, malaria remains, 120 years after *Plasmodium* discovery, one of the major health problems, particularly in Africa. Among the different strategies used, vector control is an important component of malaria control. Insecticide-treated nets (ITNs) and indoor residual spraying (IRS) represent the front-line tools for malaria vector control. However as a real arms race, anophelines mosquitoes develop more and more resistance. The aim of this study was to identify changes in the principal malaria vectors in Dielmo (Senegal) that occurred before (2006 to July 2008) and after (August 2008 to December 2011) the implementation of LLIN's. Adult mosquitoes were collected by HLC monthly from January 2006 to December 2011 and by PSC during the rainy season. Mosquitoes were identified down to their species and sub-species by PCR. The presence of circumsporozoite protein (CSP) of *P. falciparum* and the blood meal origin was detected by ELISA, and kdr mutations were investigated by PCR. From January 2006 to December 2011, 855 (62.0%) *An. gambiae s.l.* and 5,190 (36.3%) *An. funestus* were collected during 744 man night captures. No *An. gambiae* HBR variations was observed during periods before and after the implementation of LLINs (12.0 An./man/night vs. 11.8 before), whereas *An. funestus* present all year round before LLINs disappeared after. Before the implementation of LLINs, CSP rate was not significantly different between kdr groups (4.55% in RS group and 3.83% in SS group, p=0.6) or in taxa groups (p=0.6). After August 2008, a significant difference was observed among kdr groups with significantly more CSP positive specimens in the RR group (25.45%) than in RS (14.67%) or SS groups (p<0.001). A significant difference in CSP rate was also observed between taxa groups (p=0.004) with more infected mosquitoes in *An. gambiae* S form. No specimen with L1014S kdr mutation was identified along the study. This study demonstrates the exceptional adaptability of *An. gambiae s.l.* to the presence of insecticide inside the houses.

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CHANGES IN *ANOPHELES FUNESTUS* BITING BEHAVIOR FOLLOWING UNIVERSAL COVERAGE OF LONG-LASTING INSECTICIDAL NETS IN BENIN

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Behavioural modification of malaria vectors in response to vector control methods is of great concern. We investigated whether full coverage of Long-Lasting Insecticide-treated mosquito Nets (LLIN) may induce a switch in biting behaviour in *Anopheles funestus*, a major malaria vector in Africa. Human landing collections were conducted indoor and outdoor in two villages (Lokohouè and Tokoli) in Southern Benin prior, 1 year and 3 years after implementation of universal LLIN coverage. Outdoor Biting Rates (OBR) and Median Catching Times (MCT, the hour for which 50% of *An. funestus* mosquitoes were collected) were compared. The resistance status of *An. funestus* to deltamethrin insecticide was monitored using bioassays. MCT of *An. funestus* switched from 02:00 in Lokohoué and 03:00 in Tokoli to 05:00 after 3 years (Mann-Whitney p-value<0.0001). In Tokoli, OBR increased from 45% to 68.1% (OR=2.55;95CI 1.72-3.78;p<0.0001) 1 year after the universal coverage whereas OBR was unchanged in Lokohoué. In this latter place, however, the proportion of *An. funestus* that bites after dawn (06:00) was 26%. Bioassays showed no resistance to deltamethrin. In conclusion, this study provides evidence for a switch in malaria vectors biting behaviour following the implementation of LLIN at universal coverage. We show first evidence for a diurnal activity of a major malaria vector in Africa. These changes may reflect phenotypic plasticity or selection of genetically inherited traits and may have direct consequences on the burden of malaria in Africa. These findings highlighted the need for alternative strategies for better targeting outdoor malaria vectors.

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QUANTIFYING THE MOSQUITO'S SWEET TOOTH: MODELING ATTRACTIVE TOXIC SUGAR BAIT FOR VECTOR CONTROL

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Current vector control strategies focus largely on indoor measures, such as long-lasting insecticide treated nets (LLINs) and indoor residual spraying (IRS); however mosquitoes frequently feed on sugar sources outdoors, inviting the possibility of novel control strategies. Attractive toxic sugar bait (ATSB), either sprayed on vegetation or provided in outdoor bait stations, has been tested in Mali and Israel and has been shown to significantly reduce mosquito densities in these settings. We fitted models of mosquito population dynamics to data from these experiments to gain a better quantitative understanding of mosquito sugar feeding behavior and the potential of ATSB to control mosquito populations. In Mali, we estimate that 42% of female mosquitoes in the experimental setting fed on ATSB solution per day, dying within three hours of ingesting the toxin. A model incorportating the number of gonotrophic cycles completed by female mosquitoes found a higher feeding rate for younger mosquitoes and a slower rate for older mosquitoes.This model was extended to assess the role of ATSB as part of an integrated vector management (IVM) program. Our simulations suggest that an IVM program based on on both ATSB and LLINs/IRS is likely to cause substantial reductions in mosquito density as multiple stages of the mosquito's lifecycle are targeted. In addition, ATSB is expected to be particularly effective against *Anopheles* arabiensis, which is relatively exophilic and therefore less affected by IRS and LLINs. ATSB has a benefit over larvacides in the sense that it skews the age distribution towards younger mosquitoes, which is beneficial for malaria control because only older mosquitoes have time to acquire, incubate and transmit the parasite. These encouraging results suggest that ATSB should be seriously considered as a promising component in future IVM malaria control strategies.

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A POTENTIAL ROLE FOR URIC ACID IN ENDOTHELIUM ACTIVATION AND DAMAGE IN *PLASMODIUM FALCIPARUM* MALARIA

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Inflammatory cytokinemia and systemic endothelial activation are central to the pathogenesis of *Plasmodium falciparum* (Pf) malaria. Recently, Pf-derived uric acid (UA) - in both its soluble and precipitated forms - was shown to activate human immune cells *in vitro*, and elevated plasma UA levels were associated with inflammatory cytokinemia and disease severity in children with malaria. A role for Pf-derived UA in endothelial inflammation has not been investigated. Since UA elevations are associated with endothelial inflammation in a variety of non-malarial diseases, we hypothesized that elevated UA levels contribute to endothelial activation and damage in *P. falciparum* malaria. To test this, we measured levels of UA and soluble forms of intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), E-Selectin, thrombomodulin (TM), tissue factor (TF) and vascular endothelial growth factor (VEGF) in the plasma of 567 Malian children (aged 6 months - 17 years) with uncomplicated malaria (UM, N=489) and non-cerebral severe malaria (NCSM, N=68). In 69 of these children, we measured these same factors during their malaria episode and twice when they were healthy (before and after the transmission season). We found that levels of UA, sICAM-1, sVCAM-1, sE-Selectin and sTM increased significantly during a malaria episode, returning to 'healthy' levels at the end of the transmission season ($p<0.0001$). In children with UM, UA levels correlated significantly with those of sICAM-1 ($r=0.255$, $p<0.0001$) and sTM ($r=0.175$, $p=0.0005$). To test the possibility that Pf-derived UA precipitates activate EC, we co-cultured 3D7-infected red blood cells (PfRBCs) with primary microvascular endothelial cells (MVECs) with or without uricase, which degrades UA. Our preliminary results show that PfRBCs stimulate MVECs to release IL-6 and IL-8 in a dose- and time-dependent manner and that uricase abrogates the production of these cytokines. Our data suggest that parasite-induced elevations in UA levels contribute to malaria pathogenesis by causing endothelial activation and damage

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GLIDING MOTILITY AND ERYTHROCYTE INVASION PROCESSES OF *BABESIA* MEROZOITES VISUALIZED BY TIME-LAPSE VIDEO MICROSCOPY

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Babesiosis is a zoonosis caused by tick-transmitted intraerythrocytic protozoa of the Phylum Apicomplexa. Some specific stages of apicomplexan parasites, such as sporozoites of *Plasmodium falciparum* and tachyzoites of *Toxoplasma gondii*, invade their target host cells using a unique, active process known as gliding motility. However, it is not thoroughly understood how the merozoites of *Babesia* parasites target and invade the host red blood cells (RBCs), and the gliding motility has so far not been observed in the parasite. In this study, we revealed the gliding motility of *B. bovis* merozoites by time-lapse video microscopy. The recorded images delineated that the processes included egress of the merozoites from the infected RBC, gliding motility, and succeeding invasion into new RBCs. Based on these images, the gliding motility of *B. bovis* merozoites was similar to the helical gliding of *Toxoplasma* tachyzoites. The trails left by the merozoites were detected by indirect immunofluorescence assay using antiserum against *B. bovis* merozoite surface antigen 1. This first report of gliding motility in *B. bovis* is notable and significant for the apicomplexan parasites since merozoites of *Plasmodium* parasites do not glide on the substrate. Furthermore, inhibition of gliding motility by actin filament polymerizer or depolymerizer indicated that this movement was driven by actomyosin-dependent process. In this study, we also revealed the timing of breakdown of parasitophorous vacuole through time-lapse image analysis. The membrane-stained bovine RBCs showed formation and breakdown of parasitophorous vacuole within ten minutes. Moreover, recent studies in *Plasmodium* have highlighted the essential role of the thrombospondin related anonymous/adhesive protein (TRAP) family in the gliding and cell invasion of the parasites. Currently, we are in the process of investigating the role of the TRAP-family in the gliding motility of *Babesia* merozoites.

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THE ROLE OF MACROPHAGES IN SCHISTOSOMAL BLADDER PATHOGENESIS

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Schistosoma haematobium infects 112 million people, rendering it the most prevalent cause of schistosomiasis worldwide. Chronic *S. haematobium* infection (urogenital schistosomiasis) leads to approximately 150,000 deaths annually from urinary tract fibrosis-induced obstructive renal failure, making it one of the deadliest worm infections globally. Despite the major human impact of urogenital schistosomiasis, the mechanisms of *S. haematobium* egg-triggered, urinary tract granuloma-associated pathogenesis remain ill-defined. Parallels may be drawn from mouse models of *Schistosoma mansoni* infection, wherein egg granuloma-associated macrophages play a central role in liver pathogenesis. However, the involvement of macrophages in schistosomal bladder pathogenesis is unknown. To address this deficiency, we have employed our recently developed mouse model of urogenital schistosomiasis, as reported previously. Eight to 12 week old female mice underwent bladder wall microinjection with various single doses of *S. haematobium* eggs. Macrophages were systemically and locally killed by intraperitoneal, bladder intramural, and transurethral administration of macrophage-

depleting agents. Serial microultrasonography revealed zones of decreased echogenicity in the periphery of egg granulomas in macrophage-depleted versus -replete mice, suggestive of relative hypocellularity. This was confirmed by histology, which revealed hypocellular cavitations in macrophage-depleted granulomas, less fibrosis, and fewer infiltrating leukocytes. None of the control vehicle-treated mice receiving eggs died, whereas 60% of macrophage-depleted mice receiving high doses of eggs died by day 11 post-egg injection, indicating a crucial role for macrophages in prevention of detrimental systemic effects of helminth exposure. Our results confirm a critical role for macrophages in schistosomal bladder pathogenesis, even in the setting of a single exposure to *S. haematobium* eggs. This suggests that macrophages may be a suitable therapeutic target for advanced schistosomal bladder pathogenesis.

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DEVELOPMENTAL AND SPATIAL EXPRESSION OF ANTIGENIC GLYCANS BY LARVAL STAGES OF *SCHISTOSOMA MANSONI*

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Many glycans of schistosomes are differentially expressed during the parasite's lifecycle. In human infections, antibodies are produced against antigenic schistosome glycans, which might form promising novel vaccine targets. Schistosome larvae, up to several days old, appear most vulnerable to immune attack, but their glycosylation is poorly characterized. In this study we determined the structure and localisation of the antigenic glycans expressed by early developmental stages of *S. mansoni* ranging from invading cercariae to transformed schistosomula and mature worms, with the objective of identifying antigens exposed to the immune system of the host. The protein-linked glycans from 14 different lifecycle stages were isolated and profiled using a mass spectrometry-based analysis strategy. Although N-glycans were continuously present during the whole lifecycle, our analysis indicated a gradually changing N-glycome during development. Expression of immunogenic glycan elements such as core-xylose and LeX-antennae are abundant in cercariae and shortly after transformation to schistosomula, but expression decreased after 3 days of maturation. On the other hand, glycans with LDN termini become abundant in the adult stages. O-glycan expression, often with similar antennae motifs as N-glycans, strongly diminishes after transformation of the cercariae, but becomes abundant again in eggs. Using a glycan-microarray constructed of schistosome-derived glycans, we determined the fine-specificity of a panel of anti-carbohydrate monoclonal antibodies obtained from schistosome-infected mice. Application of these mAbs in immunofluorescence microscopy assays of the infective cercariae and 1-3 day schistosomula stages of *S. mansoni* indicated that some glycan epitopes (e.g. LDN, F-LDN, F-LDN-F) identified in the structural studies are expressed at the surface throughout development, whereas others such as the LeX-motif appear at the surface only after transformation. These observations further underline the potential of specific glycans as targets for immune attack.

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NEMATODE AUTOPHAGY REGULATES *WOLBACHIA* POPULATIONS AND IDENTIFIES A NOVEL MODE-OF-ACTION FOR ANTI-FILARIAL TREATMENT

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Filarial nematode parasites are amongst the most important neglected parasitic diseases of humans and animals. Over 150 million individuals are infected with lymphatic filariasis and onchocerciasis and heartworm is an important pathogen of dogs and cats. A new target for anti-filarial treatment is the obligate mutualistic endobacteria *Wolbachia*. Depletion of *Wolbachia* with antibiotics induces defects in nematode development, fertility and viability. In order to identify novel mechanisms to deplete *Wolbachia* as part of the A-WOL drug discovery and development programme, we investigated the role of activating host nematode autophagy to control bacterial populations. Our studies revealed that periods of rapid bacterial population growth and expansion were accompanied by activation of the autophagy pathway and that chemical and genetic manipulation of this pathway could directly regulate bacterial populations at an equivalent level to antibiotic treatment. The activation of the autophagy by using drugs or small-molecules resulted in *Wolbachia* reduction in both *in vitro* and *in vivo* treated *Brugia malayi*. The induction of the host nematode intracellular autophagy defence mechanism can therefore be considered as a novel mode-of-action, which delivers bactericidal activity that can be used to develop improved drugs and regimes for anti-filarial treatment.

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FUNCTIONAL REDUNDANCY IN *PLASMODIUM* HEMOGLOBINASES AND PARASITE DEVELOPMENT INSIDE RETICULOCYTES WITHOUT HEMOGLOBIN DEGRADATION

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Clinical symptoms of malaria infection manifest when the *Plasmodium* parasites replicates in host red blood cell. During this intraerythocytic cycle, the parasite ingests and catabolizes up to 75% of the host cell homoglobin (Hb). The hemoglobin is broken down by a number of proteases in a semi-order cascade in an acidic, *Plasmodium*-specific, digestive food vacuole (DFV). Whether this degradation is essential for parasite survival has not been established. To characterize biological roles of various hemoglobinases residing in digestive food vacuole, we attempted targeted gene disruption of all the predicted hemoglobinases in the rodent model P. berghei and found that most of them are functionally redundant. We have also created a double gene-deletion mutant, Δpm4Δbp2, lacking expression of both plasmepsin-4 (equivalent to *P. falciparum* plasmepsin I, II, III and IV) and bergheipain-2 (equivalent to *P. falciparum* falcipain-2a, 2b and 3), which are thought to be involved in initial cleavage of Hb. Despite severe growth and virulence attenuation, the parasites are able to develop into mature schizonts in reticulocytes. These schizonts produce either no or vastly reduced levels of hemozoin, the crystallized product formed by detoxification of heme that is released early in Hb digestion. This was confirmed by examining hemozoin deposition in both liver and spleen of Δpm4Δbp2 infected mice, which was greatly reduced compared to wildtype infected mice. The cerebral complication (CM) sensitive C57BL/6 mice were able to clear the infection without visible CM manifestation and survive later wild-type challenge. Our results show that Hb digestion may not be essential for parasite growth in reticulocytes. These findings have implications for the design of drugs against DFV enzymes and for possible mechanisms that underlie *Plasmodium* resistance to drugs, the majority of which target Hb digestion and heme detoxification.

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BIOCHEMICAL CHARACTERIZATION OF UFSP, THE UFM1 ASSOCIATED PEPTIDASE IN *LEISHMANIA DONOVANI*

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Leishmaniasis is a spectrum of diseases caused by protozoan parasites belonging to several different *Leishmania* species. There are no effective

vaccines against leishmaniases. Currently available therapeutic regimens are often limited in effectiveness due to unwarranted side effects and rapidly emerging drug resistance. Therefore, the quest for a novel vaccine and therapeutic targets acquires urgency towards controlling leishmaniases. Ubiquitin and ubiquitin like protein modifiers (Ubls) regulate a variety of biological functions ranging from endocytosis, membrane trafficking, protein kinase activation, DNA repair and chromatin dynamics. Studies of Ubl functions in human parasitic organisms are limited. Recently, we described the existence of a novel Ubl named ubiquitin-fold modifier 1 (Ufm1) that conjugates to parasite proteins in *Leishmania donovani*. To elucidate the enzymatic activities associated with Ufm1 conjugation, we identified a putative Ufsp in the trypanosomatid genomes. Biochemical analysis of *L. donovani* Ufsp showed that this protein possesses the 3'hydrolase activity necessary for processing the precursor Ufm1 into a conjugatable form. To examine the effects of abolition of Ufm1 processing activity, we generated a *L. donovani* knock out mutant lacking the Ufsp. Analysis of the Ufsp mutant revealed that lack of this protein results in the absence of processing of precursor Ufm1. We also showed that Ufsp null mutant results in reduced survival of *L. donovani* in infected human macrophages suggesting a role for this protein in *Leishmania* pathogenesis. This growth defect was reversed by re-expression of wild type but not the mutant of the catalytic cysteine (cys>ser) in the Ufsp indicating the essential nature of this protease for Ufm1 conjugation reactions. Therefore, *Leishmania* Ufsp has the potential to be a novel drug target. Further, Ufsp-/- parasites also provide an opportunity to explore such parasites as live attenuated vaccine candidates.

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MOLECULAR AND FUNCTIONAL STUDIES OF THE *SCHISTOSOMA MANSONI* VENUS KINASE RECEPTORS SMVKR1 AND SMVKR2: POTENTIAL ROLES IN LARVAL DEVELOPMENT AND OOGENESIS

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Venus kinase receptors (VKRs) form a new family of receptor tyrosine kinases. Atypically, VKRs contain an extracellular Venus Flytrap (VFT) domain, a ligand-binding domain activated by small molecules such as aminoacids. *Vkr* genes are found in diverse eumetazoan genomes, from cnidarians to echinoderms and are particularly well conserved in protostomian species, as reported previously. In the platyhelminth *Schistosoma mansoni*, two VKRs have been previously described, SmVKR1 and SmVKR2. Quantitative RT-PCR as well as *in situ* hybridization indicated a large expression of both genes in larval stages and in female ovaries. RNA interference experiments performed on sporocysts and adult worms further confirmed the implication of SmVKRs in larval development and oogenesis. Using *Xenopus laevis* oocytes for protein expression, we demonstrated that SmVKR1 could bind and be activated by amino acids, mainly by L-Arginine, whereas SmVKR2 activation was triggered by calcium ions. In order to decipher the downstream signalling pathways of SmVKR1 and SmVKR2, we have started to identify binding partners of these receptors by the screening of an adult worm cDNA library using the yeast two-hybrid system. Our results suggest that both SmVKR1 and SmVKR2 participate in cytoskeleton rearrangement and in developmental mechanisms. Potential substrate/adapters for SmVKR1 have been identified and their function in the activation pathway of the receptor is under investigation.

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DETECTING CRYPTIC GENETIC EXCHANGE IN *TRYPANOSOMA CRUZI* TCI

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Clonal propagation is considered to be the predominant mode of reproduction among many parasitic protozoa. However, this assumption may overlook unorthodox, infrequent or cryptic sexuality. *Trypanosoma cruzi*, the causative agent of Chagas disease, is known to undergo non-Mendelian recombination *in vitro*, while evidence of natural genetic exchange is more limited. *T. cruzi* displays remarkable genetic heterogeneity and is recognized as a complex of six discrete typing units (DTUs). Infection with *T. cruzi* is life-long and can lead to a spectrum of pathological complications. However, the relationship between specific clinical outcomes and parasite genotype remains elusive. The availability of whole genome sequences has driven advances in high-resolution genotyping techniques and re-invigorated interest in exploring the genetic diversity present within the various DTUs. We developed a highly resolutive mitochondrial multilocus sequence typing (mtMLST) scheme, which was evaluated against current nuclear typing tools using isolates belonging to the oldest and most widely occurring lineage TcI. In parallel, we exploited read depth data, generated by Illumina sequencing of the mitochondrial genome from the TcI reference strain to investigate the existence of mitochondrial heteroplasmy (heterogeneous mitochondrial genomes in an individual cell) in *T. cruzi* and resolve its role as a potential source of genotyping error. Comparison of nuclear and mitochondrial data uncovered multiple novel mitochondrial introgression events among disparate geographical populations as well as between major *T. cruzi* DTUs. Illumina sequencing data from the TcI genome strain revealed multiple different mitochondrial genomes within an individual parasite (heteroplasmy) that were, however, not sufficiently divergent to represent a major source of typing error. mtMLST provides a powerful approach to genotyping at the sub-DTU level. This strategy will facilitate attempts to resolve phenotypic variation in *T. cruzi* and to address epidemiologically important hypotheses in conjunction with intensive spatio-temporal sampling. The observations of gross nuclear-mitochondrial phylogenetic incongruence indicate that genetic recombination is geographically widespread and continues to influence the natural population structure of TcI, a conclusion which challenges the traditional paradigm of clonality in *T. cruzi*.

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A *PLASMODIUM VIVAX* GENETIC CROSS TO INVESTIGATE MOLECULAR DETERMINANTS OF CHLOROQUINE RESPONSE

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To investigate determinants of *Plasmodium vivax* chloroquine (CQ) resistance we have generated a genetic cross between two parasite lines with distinct CQ responses. A chimpanzee was infected with a mixture of these parental lines to produce infectious gametocytes for cross-fertilization in *Anopheles* mosquitoes. Recombinant sporozoites from the infected mosquitoes were purified and cryopreserved and subsequently used to re-inoculate the same chimpanzee after it was completely cured of the parental lines infection. When parasitemia was detected in the

re-inoculated chimpanzee, pools of mixed intraerythrocytic recombinant progeny were collected and inoculated into *Aotus* monkeys. Progeny in these pools showed responses spanning the range of the parental lines, including some parasites surviving a total CQ dose of 15 mg/kg (5 mg/kg/day x 3 days). Comparison of genetic markers in the mixed progeny before and after CQ treatment identifies regions of chromosomes that may be subject to linkage group selection and contain possible candidate genes. The *P. vivax* ortholog of the *P. falciparum* CQ resistance transporter gene (*pfcrt* ortholog, *pvcrt*-o) resides in one of these chromosome regions.

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ARTEMISININ RESISTANCE IN *PLASMODIUM FALCIPARUM* IS ASSOCIATED WITH AN ALTERED PATTERN OF TRANSCRIPTION

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The emergence of artemisinin resistance in Western Cambodia and spread of resistance evidenced by the recent report of resistance in neighboring Thai-Myanmar border are major obstacles to the global containment and elimination of malaria disease. Although several genome-wide association studies on artemisinin resistance have been carried out proposing candidate genes, yet no definite molecular makers of artemisinin resistance have been commonly identified by the various research groups and none validated so far. Using DNA microarrays, we characterized the transcriptional profile of the ex-vivo intra-erythrocytic stage of total 36 parasite isolates from patients collected from 2008 to 2010 from Laos, Pailin, Western Cambodia and Thai-Myanmar border, of which 15 are resistant to artemisinin as reflected by increased parasite clearance half-lives. Features of the profile associated with artemisinin resistance include reduced expression of metabolic and cellular pathways such as glycolysis, pentose phosphate pathway, protein sythesis, DNA replication and redox metabolism in early stages. In contrast, protein synthesis related functions including cytoplasmic translation, transcription and chaperone-assisted protein folding genes have increased expression in the schizont stage. Hence, artemisinin resistance may be associated with lower metabolic ativity of the ring stage that leads to decreased drug activation and simultaneously, increased protein syntheis, folding and turnover, that compensate the loss of proteins damage caused by the drug. In addition, we observed the differential expression of several key regulatory proteins that may underline the observed transcription profile. The transcriptional profiles of a further 73 samples including 53 Pailin, 18 Lao and 2 Thai isolates from the ongoing TRAC (Tracking Resistance to Artemisinin Collaboration) study has been generated and analyzed and results will be discussed. In order to identify CNVs associated with resistance, we performed comparative genomic hybridizations using genomic DNA sequentially isolated from the same clinical samples and found several genes with copy number variations (CNV) associated with increased clearance half life. The involvement of these CNVs in resistance as well as their relation to the differential transcriptional profile associated with resistance phenotype will be reviewed and discussed.

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ADAPTIVE EVOLUTION OF A RING UBIQUITIN LIGASE MEDIATES REDUCED DRUG SENSITIVITY IN *PLASMODIUM FALCIPARUM*

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The main obstacle to the eradication efforts of *Plasmodium falciparum* is the parasite's genome plasticity enabling adaptation to selective pressure exerted by its human host. This has led to the lack of vaccines inducing sterile immunity and a growing dilemma of resistance to existing antimalarials. Recent population genetics approaches have revealed several regions of the genome to be under positive selection, thereby providing candidate loci needed to be scrutinized for their role in parasite biology. A surprisingly large number of the encoded molecules are putatively involved in ubiquitylation arguing that post-translational modification through ubiquitylation is a major pathway for parasite adaptation. We have here characterized a RING ubiquitin ligase (PFF1325c) with one non-synonymous SNP (D113N) under recent positive selection. Recombinant wild type and mutant protein was expressed and were both shown to mediate formation of ubiquitin chains in reactions with human conjugating enzymes UbcH5a-c. This proves PFF1325c to be a true ubiquitin ligase and gives important clues to which conjugating enzymes are likely interactors within the parasite. To directly assess the influence of the D113N mutation on parasite biology, we introduced the two different allelic variants into different parasite genomes. No difference in growth was detected for the allelic variants under normal *in vitro* growth conditions. However, a clear shift in IC_{50} to chloroquine (CQ) and amodiaquine (ADQ) was observed, with parasites carrying the mutant allele being less sensitive. To capture the evolutionary benefits of the mutant allele under drug pressure, parasite clones were matched in competition experiments with or without CQ and ADQ. Parasites carrying the mutant allele clearly outcompeted their wild type counterparts at sub-lethal drug concentrations and recrudesced faster after exposure to lethal concentrations of drug. Our data suggest modification of the ubiquitylation cascade to be an important adaptive response and a novel contributor to drug resistance in *P. falciparum*.

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EX VIVO ANTIMALARIAL DRUG SUSCEPTIBILITY OF *PLASMODIUM FALCIPARUM* IN WESTERN, NORTHERN AND EASTERN CAMBODIA, 2011

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Artesunate (ART) plus mefloquine (MQ) was introduced as first-line treatment for *Plasmodium falciparum* malaria in Cambodia in 2001. In 2009, *P. falciparum* resistance to ART+MQ was reported in Western Cambodia, prompting the National Malaria Control Program to recommend dihydroartemisinin (DHA) + piperaquine (PPQ) for this region. In recent years, however, there have been few reports on the *ex vivo* susceptibility of *P. falciparum* to these and other antimalarial drugs in W. Cambodia or elsewhere in the country. To establish profiles of *ex*

vivo antimalarial drug susceptibility in W. Cambodia, and compare them with those in Northern and Eastern Cambodia, we obtained *P. falciparum* isolates directly from patients with uncomplicated malaria. Using a SyBR-Green I-based method, we measured the *ex vivo* susceptibility of 252 parasite isolates to 6 antimalarial drugs: chloroquine (CQ), MQ, quinine (QN), PPQ, ART and DHA. Data from 80% (203/252) of these assays were interpretable for ≥ 4 drugs. The proportions of parasite isolates showing reduced *ex vivo* susceptibility to CQ, MQ, QN and PPQ in W. Cambodia were 98%, 22%, 4% and 10%, respectively. The same proportions in N. Cambodia were 97%, 20%, 7% and 7% and in E. Cambodia were 84%, 5%, 0% and 10%. Reduced *ex vivo* susceptibility to ART and DHA was not observed in the 3 regions. The *ex vivo* mean IC_{50} (GM IC_{50}) values for CQ, MQ, QN, ART and DHA were significantly higher in W. and N. Cambodia than in E. Cambodia (p<0.001). However, there were no significant differences in the *ex vivo* GM IC_{50} values for PPQ between these regions. We detected significant positive correlations between MQ and ART (r=0.54, p<0.001), MQ and DHA (r=0.32, p<0.001), QN and ART (r=0.62, p<0.001) and QN and DHA (r=0.42, p<0.001). Our data indicate that reduced *P. falciparum* susceptibility to MQ and PPQ is present in all 3 regions of Cambodia. In different regions of Cambodia, where either DHA-PPQ or ART+MQ are the recommended treatments, studies to monitor the clinical efficacy of these drugs is warranted.

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DEVELOPMENT OF ARTESUNATE RESISTANCE *IN VIVO* USING A *PLASMODIUM FALCIPARUM* HUMANIZED MURINE MODEL OF MALARIA

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Development of resistance against artemisinin-combination therapies (ACTs) is a major threat for the control and eradication of malaria. Humanized murine models of malaria allow the growth of *Plasmodium falciparum* in human erythrocytes engrafted into mice. In this work we show that the *P. falciparum* murine model can be used to analyze the development of resistance against antimalarials. Treatment failure with artesunate was observed after suboptimal therapy in thirteen serial passages whereas atovaquone required two, which is compatible with their corresponding propensity to generate resistance. The artesunate resistant strain showed a marked decrease in the parasite reduction ratio (PRR) whereas the atovaquone resistant strain showed almost complete resistance to treatment *in vivo*. None of the resistant strains showed measurable impairment of proliferative capacity *in vivo*. Interestingly, in contrast with atovaquone, the reduction of susceptibility to treatment with artesunate was not associated with reduced susceptibility *in vitro*. Therefore, these results suggest that the *P. falciparum* humanized model can be a valid model to study the development of resistance caused by sub-therapeutic treatment.

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ARTEMETHER-LUMEFANTRINE SELECTS FOR MALARIA PARASITES WITH DECREASED LUMEFANTRINE SENSITIVITY ALTHOUGH PARASITES REMAIN SENSITIVE TO THIS REGIMEN IN TORORO, UGANDA

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Artemisinin-based combination therapies (ACTs) may select for malaria parasites with decreased drug sensitivity. We studied the sensitivity of parasites from children enrolled in treatment and prevention trials in Tororo, Uganda from June, 2010 to February, 2012. When *Plasmodium falciparum* malaria was diagnosed, blood was obtained, parasites (286 isolates) were cultured with serial dilutions of chloroquine (CQ), monodesethylamodiaquine (AQ), quinine (QN), dihydroartemisinin (DHA), lumefantrine (LM), or piperaquine (PQ) for 72 h, and *ex vivo* sensitivities were assessed by HRP-2-based ELISA. Sensitivities (nM) to CQ (median IC50 486.2; IQR 206.5-748.8), AQ (83.3; 58.4-132.4), PQ (20.3; 7.6-47.5) and QN (126.4; 74.9-196.3) varied widely; parasites were highly sensitive to LM (2.7; 0.97-6.7) and DHA (1.7; 1.0-2.8). IC50 values for 4 successive quartiles, each with 71-73 isolates collected over ~4 months, varied little for control strains (3D7 IC50s 0.54-1.8 nM) and all drugs except LM and PQ. For PQ, sensitivity decreased after the first quartile (median IC_{50} 6.5), but was then stable (26.6-32.0). For LM, IC_{50}s were low, but increased consistently (successive quartiles 1.1, 2.3, 3.5, and 6.2, p<0.05 for all comparisons except 2nd-3rd quartiles). Receiving monthly DHA-PQ in a prevention trial was not associated with changes in PQ sensitivity compared to those receiving placebo. Having received artemether-LM (AL) within 60 days as treatment for a prior episode of malaria was associated with decreased sensitivity to LM (median IC_{50} 1.59 for those without [n=84] vs. 3.24 for those with [n=119] recent AL; p=0.022 [GEE regression with log IC50 values]), but no significant change in sensitivity to the other study drugs. In summary, recent isolates of *P. falciparum* in Tororo were highly sensitive to components of AL, the national treatment regimen, but treatment with AL selected for parasites with decreased sensitivity, and overall sensitivities decreased from 2010 to 2012. Longitudinal surveillance of sensitivities of parasites under different selective drug pressures continues.

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WHAT DETERMINES PARASITE CLEARANCE: A POOLED ANALYSIS OF FREQUENT PARASITE COUNTS AFTER TREATMENT WITH AN ARTEMISININ DERIVATIVE ALONE OR IN COMBINATION WITH OTHER ANTIMALARIALS

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Parasite clearance (PC) is considered to be the most robust measure of antimalarial effect and is a key component to characterize artemisinin resistance. The dynamics of PC following artemisinin treatment are influenced by several factors other than parasite susceptibility including host immunity, initial parasite biomass, and partner drug efficacy. It is critically important to control for such potential confounding factors to identify changes over time in PC due to reduced parasite drug susceptibility. We have pooled parasitaemia data collected at least every 12 hours from individual patients who participated in completed studies. The WWARN Parasite Clearance Estimator was used to produce standardized estimates of parasite half-life (HL). The effects of covariates such as artesunate dose, partner drug, transmission intensity, year and location of study, age and baseline characteristics on PC were examined in a regression model and the relationship between treatment outcome and HL was evaluated in a subset of patients with efficacy data available using Cox regression. Random effects or frailty were used to account for study effect. Fourteen studies with 4655 patients in Cambodia, Thailand, Laos, Bangladesh, Mali, Tanzania and Kenya were included in the analysis. Clinical outcome was evaluated in 8 studies with 702 patients during 42 (4 studies) or 63 days (4 studies) follow-up period. The median (range) of estimated HLs was 3.2h (0.6 - 21.4). Estimates varied significantly between study location and year ($p<0.001$), with median HLs ranging 1.9-6.9 h between studies. Among 696 patients with available efficacy outcomes, twenty four had PCR-confirmed recrudescence and slower PC ($p<0.001$) with a median (range) HL of 6.8h (2.5-11.1) compared to 3.3h (0.9-12.2) in cured patients. HL was not affected by initial parasite count or patient age but was longer in patients with gametocytes, low haematocrit or prolonged fever at enrollment. This analysis provides key reference baseline data to characterize antimalarial resistance and understand factors affecting measurement of PC.

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DEFINING THE ELUSIVE ARTEMISININ RESISTANCE PHENOTYPE *IN VITRO*

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Artemisinin resistance has emerged in Cambodia and Thailand and is observed clinically as a reduced parasite clearance rate *in vivo* following treatment with an artemisinin derivative alone or in combination. Recent evidence suggests the *in vivo* phenotype is linked to heritable genetic trait(s), yet to date a clear artemisinin resistance phenotype *in vitro* has not been defined. This is in direct contrast to experience with other antimalarial drugs where *in vitro* drug resistance was clearly evident in cultured parasites either before or simultaneously with the advent of clinical resistance. Through a series of studies we have begun to define the elusive artemisinin resistance phenotype *in vitro*. First we generated stable artemisinin resistant lines of *Plasmodium falciparum*, cloned them, and used these clones to assess new *in vitro* phenotype assays. Secondly we applied the new assays to culture adapted isolates of *P. falciparum* from Cambodia and Thailand. Isolates with evidence of artemisinin resistance *in vitro* were immediately cloned and characterized. Results from these studies suggest that both the *in vitro* generated resistant lines and clones of Cambodian *P. falciparum* express stable resistance to artemisinin derivatives *in vitro*. Interestingly, the highest level of resistance in all resistant lines was to artelinic acid (AL), a compound that has never been used clinically. We found 4-8 fold resistance to AL in each of the resistant lines as compared to 3-5 fold resistance to artemisinin. A reduced level of resistance (2-3 fold) was consistently observed for dihydroartemisinin. In addition, each of the artemisinin resistant lines expressed the artemisinin-induced ring stage dormancy phenotype in which the resistant line recovered more rapidly from dormancy than artemisinin susceptible parasites. These new artemisinin resistance phenotypes can be used to monitor emerging resistance in the field and to accelerate the discovery of drug resistance mechanism(s) in stable, culturable, clonal lines.

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ASSOCIATION BETWEEN ANTIBODIES TO *PLASMODIUM FALCIPARUM* AT DELIVERY AND IMPROVED PREGNANCY OUTCOMES AMONG WOMEN EXPOSED TO MALARIA

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Antibodies against VAR2CSA, the variant surface antigen binding to placental chondroitin sulfate A, have been suggested to mediate protection against *Plasmodium falciparum* in pregnancy, although some studies have indicated that these antibodies at delivery are markers of exposure to *P. falciparum*. We hypothesized that variations in parasite exposure and HIV infection affect levels of antimalarial antibodies and also their associations with pregnancy outcomes. We measured IgGs against placental and pediatric isolates, VAR2CSA (DBL2X, DBL3X, DBL5ε, DBL6ε) and other blood stage antigens (DBLγ, DBLα, MSP1, AMA1, EBA175) in plasmas from 293 Mozambican pregnant women at delivery. The number of antigens recognized by IgG in plasma (breadth of recognition) was higher in women with placental infection (adjusted rate ratio [aRR]=1.59, 95%CI[1.44-1.77]), in women living close to the river (aRR=1.16, 95%CI[1.02-1.32]), in HIV-infected women pregnant during the rainy season (aRR=1.82, 95%CI[1.15-2.86]) and in HIV-infected women not receiving intermittent preventive treatment (IPTp; aRR=1.39; 95%CI [1.1-1.72]). HIV-infection attenuated the parity-dependent increase of IgGs against placental and pediatric isolates, DBLγ and AMA1 (p for interaction between HIV and parity≤0.046). Among women who had a malaria episode during pregnancy, high antibody level against VAR2CSA (DBL3X and DBL6ε), placental and paediatric isolates and AMA1 were associated with increased weight and gestational age of the newborns (p≤0.036). Anti-parasite IgGs in women at delivery are sensitive to factors influencing malaria exposure and are affected by HIV infection, probably through its impact on the longevity of antibody responses. Reducing the variability of parasite exposure by including in the analysis only women with proven exposure during pregnancy allows the identification of IgGs against merozoite antigens, VAR2CSA and other variant surface antigens that may contribute to reduce the adverse effects of malaria in pregnancy.

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CD44 IS A FUNCTIONALLY RELEVANT RECEPTOR FOR ADHERENT *PLASMODIUM FALCIPARUM* IN THE PLACENTA

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Plasmodium falciparum infected red blood cells (iRBCs) accumulate in the maternal blood space of the placenta during malaria infection, culminating in pathological consequences deleterious to pregnancy success. The fetal cell in contact with maternal placental blood is a syncytialized epithelium called syncytiotrophoblast (ST). ST has a rich supply of low sulfated chondroitin sulfate A (CSA), a principle ligand for VAR2CSA parasite protein, present on the surface of placenta-adherent iRBCs. It is critical to examine the role CSA-bearing proteoglycans on ST play in anchoring iRBCs as well as their potential role as signaling molecules. Because it is known that STs are immunologically active in the presence of CSA-adherent iRBCs, here we examined the role of CD44 proteoglycan, a known CSA-bearing molecule with a transmembrane cytoplasmic domains adept at signaling functions. STs membrane proteins (SMPs) were extracted from cultured primary cells as well as whole placental preparations. SMPs were incubated with CSA-adherent and non-adherent iRBCs; binding of CD44 was specific to CSA-adherent iRBCs as observed by flow cytometry. CD44 from SMPs pre-treated with chondroitinase ABC lost significant iRBC binding activity. *In vitro* exposure of primary ST to CSA-adherent iRBCs promoted upregulated expression of CD44 as detected by ELISA, and immunohistochemical staining for CD44 antigen in placental tissue from Kenyan women showed a significant increase in expression of this adherence receptor coincident with active placental malaria. Current efforts are exploring the activation state of CD44 following exposure of ST to CSA-adherent iRBCs, as well as the impact of CD44 knockdown by RNA interference on malarial activation of ST. In summary, this work provides evidence that CD44 proteoglycan is an *in vivo* receptor for VAR2CSA-expressing iRBCs, the expression of which is modulated by malarial infection, and may additionally serve as a signaling molecule, promoting an ST response to placental malaria.

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CHRONIC INFECTIOUS EXPOSURE DURING PREGNANCY AFFECTS NEONATAL B CELL SUBPOPULATIONS

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Chronic infections during pregnancy can expose the fetus to antigens that affect fetal B cell development. We hypothesize that resultant changes in B cell subpopulations may affect the infant's susceptibility to infection and disease. To investigate this, we examined cord blood B cell subpopulations and B cell responses to non-specific polyclonal activation in neonates with and without exposure to chronic prenatal infections. We developed six-color flow cytometry panels to differentiate subpopulations of B cells from cord blood mononuclear cells (CBMC) isolated from North American and Kenyan neonates. North American neonates had no prenatal chronic infectious exposures. Kenyan neonates examined had evidence of prenatal HIV, cytomegalovirus (CMV), P *falciparum* malaria or no infectious exposures. Proportions of B cell subpopulations were compared between the exposure groups. Additionally, we examined the ability of B cells in each group to respond to polyclonal activation in culture. We found that neonates exposed to chronic prenatal infections (HIV, CMV and malaria) displayed higher levels of atypical (CD19+CD27-CD21-IgD-) and activated (CD19+CD27+CD21-IgD-) memory B cells compared to Kenyan non-exposed and North American neonates. Little differences were appreciated in naive B cell (CD19+CD21+CD27-CD10-) or classic isotype switched memory B cell (CD19+CD27+CD21+IgD-) populations. Neonates exposed to HIV had a lower proportion of CD5+ B cell compared to all other groups. Polyclonal activation of B cells resulted in subtle shifts in CD5 and TLR2 expression, which were similar among the exposure groups. The results of our study suggest that the presence of chronic infections during pregnancy affects B cell development, leading to increased levels of atypical and activated memory B cells. The functional effects of these differences will need to be further investigated.

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THE SUPPRESSION OF MALARIA ANTIGEN-SPECIFIC RESPONSES BY REGULATORY T CELLS ACQUIRED IN UTERO PERSISTS INTO EARLY CHILDHOOD

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Prenatal exposure to malaria blood stage antigens has been associated with impaired malaria-Ag-specific Th1-type immune responses in early childhood as well as increased risk of malaria infection. Here we examined the hypotheses that tolerogenic fetal natural (CD25hi, FoxP3+CD4+) and adaptive (high IL-10) regulatory T cells develop *in utero*, and that these cells persist into early childhood, impairing T cell-assisted production of protective antibody to malaria blood stage antigens. We show that depletion of CD25hiCD4+ T cells or neutralization of IL-10 in cord blood is associated with 2.3 to >10 fold increased IFNγ production by and/or proliferation of malaria blood stage-specific lymphocytes in samples from newborns of a subgroup of malaria infected women; otherwise these newborns (classified as putatively tolerant, N=10) show weak or absent malaria Ag-driven proliferation or IFNγ production. By contrast, offspring of women uninfected with malaria (not exposed, N=24) or offspring who develop a malaria Ag-driven predominantly Th1-type response in the face of maternal prenatal malaria infection (exposed-sensitized, N=13) fail to show consistent augmentation of lymphocyte proliferation and/or IFNγ production with CD25hiCD4+ depletion and/or IL-10 neutralization. Repeat examination of these same children at 12 to 24 months of age shows persistence of these phenotypes, with putatively tolerant offspring showing an overall increased lymphocyte proliferation with CD25hiCD4+ depletion and enhanced IFNγ production with IL-10 neutralization compared to children identified as exposed-sensitized or not exposed (p= 0.006, 0.01 and p=0.02, 0.009 respectively). Thus, *in utero* exposure to malaria blood-stage antigens can induce a form of immune tolerance that is probably regulatory T cell-mediated and likely modulates malaria antigen-specific immune responses throughout early childhood.

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INTERACTIONS BETWEEN THE GENETIC DIVERSITY OF *PLASMODIUM FALCIPARUM* INFECTIONS AND BREADTH OF ANTIBODY RESPONSES IN RELATION TO IMMUNITY TO MALARIA

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In areas of high malaria transmission, the presence of asymptomatic infections with genetically diverse *Plasmodium falciparum* clones is associated with reduced risk of malaria by a yet unknown mechanism. Asymptomatic *P. falciparum* parasitemia can modify the association between antibodies to both merozoite and variant red blood cell surface antigens and the risk of malaria. Antibody responses to merozoite antigens are short-lived in the absence of continued infection. Considering these

observations and the step-wise reduction in risk of malaria with increasing breadth of antibody responses to merozoite antigens, we hypothesize that the presence of genetically diverse *P. falciparum* infections interacts with antibody responses to enhance the acquisition of immunity to malaria. To test this hypothesis, we have studied a longitudinally followed population in an area of high malaria transmission in Tanzania. A cross-sectional survey was conducted in March and April of 1999, just before the rainy season in which whole blood was collected. All the participants were monitored in the subsequent 40 weeks and episodes of malaria were recorded by a passive case detection system. We assessed the genetic diversity of *P. falciparum* infections at baseline by genotyping the *P. falciparum* merozoite surface protein 2 (*msp2*) gene by fluorescent PCR and capillary electrophoresis. We measured antibody levels to four of the leading malaria vaccine candidate antigens; 2 alleles of MSP-2, two alleles of MSP-3, two alleles of apical merozoite antigen 1, and the 19-kilodalton fragment of MSP-1 using a multiplex platform. Increasing breadth of antibody responses and presence of increasing number of genetically distinct clones at baseline were associated with reduced risk of malaria both individually and when analyzed in combination. These findings suggest that in an area of high malaria transmission, genetic diversity and antibody responses are additive or synergistic in conferring protection from malaria.

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USE OF TETRAMER STAINING TO ENUMERATE AND CHARACTERIZE MALARIA ANTIGEN-SPECIFIC CD8⁺ T-CELLS INDUCED IN VOLUNTEERS IMMUNIZED WITH ADENOVIRUS SEROTYPE 5 *PLASMODIUM FALCIPARUM* MALARIA VACCINES

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Protection against pre-erythrocytic stages of malaria is believed to be mediated in part by cytotoxic T-lymphocyte (CTL) responses against epitopes of protective antigens presented by HLA on the surface of infected liver cells. Because IFN-γ secreting CD8⁺ T cells have been implicated in protection against pre-erythrocytic stage malaria through IFN-γ-mediated induction of nitric oxide to kill intra-hepatic stage malaria parasites, it has become common practice to attempt to associate the magnitude of malaria vaccine-induced and CTL-mediated IFN-γ production with protective immunity, since antibody responses are usually poor. However, malaria antigen-specific CD8⁺ T-cells might kill infected hepatocytes by non-IFN-γ mediated mechanisms, such as the direct killing of hepatocytes through release of perforin and granzyme. In order to establish the total magnitude of the vaccine-induced CD8⁺ T cell response, we have identified putative CD8⁺ T-cell epitopes on CSP and AMA-1, using PBMC samples from volunteers immunized with a single dose of serotype 5 adenovirus expressing two malaria antigens, CSP and AMA1 (AdCA). We next prepared a series of tetramers, each consisting of one of seven HLA-A or five HLA-B class I molecules and each presenting one of 16 distinct CSP epitopes or one of 11 distinct AMA-1 epitopes. We then used the tetramer staining technique to label and thereby enumerate the total number of malaria antigen-specific CD8⁺ T cells in immunized HLA-A*01:01⁺ and HLA-B*18:01⁺ volunteers. We have also demonstrated that malaria tetramer-positive CD8⁺ T cells obtained three weeks post immunization express high levels of the CD38 and HLA-DR activation markers, raising the possibility that these markers might serve as a surrogate to detect multiple antigen-specific CD8⁺ T cells induced by whole parasite vaccines where the majority of antigens have not yet been identified. Work is also ongoing to carry out tetramer staining, ELISpot, and intracellular staining assays using pre-vaccination and post vaccination PBMC samples to look for comparability of results generated from the different assays.

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GENERATION OF NOVEL "HUMAN-IMMUNE-SYSTEM" HUMANIZED MOUSE STRAINS CO-EXPRESSING HLA CLASS I AND CLASS II MOLECULES IN NOD.RAGKO.IL2RGCKO BACKGROUND

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The current animal models that are used to test approaches that target the immune system (i.e., vaccines) are imperfect and accounts for many faileures when human vaccines are tested in clinical trials. Development of humanized mouse models able to generate a surrogate human immune system is a highly pursued goal for investigating human immunology and for testing human vaccines. We previously showed that humanized mice expressing HLA class II (DR4) molecules in NOD.RagKO.IL2RgcKO background and infused with HLA-DR-matched human hematopoietic stem cells, develop a functional human immune system and respond to vaccination (PLoS One 6:e19826, 2011). While the frequency of human CD4 T cells, B cells, and dendritic cells in blood and lymphoid organs of humanized DRAG mice was similar to that in humans, the frequency of human CD8 T cells was however lower. This was attributed to the lack of HLA class I expression in humanized DRAG mice, since HLA class I molecules are required for thymic selection and survival of human CD8 T cells. Herein we have generated a new humanized mouse strain co-expressing HLA class I (A2) and HLA class II (DR4) molecules in NOD. RagKO.IL2RgcKO background, and provide evidence for human immune cell reconstitution as well as function of human CD8 T cells upon infusion of HLA-matched human hematopoietic stem cells.

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QTL MAPPING OF *PLASMODIUM FALCIPARUM* GENES THAT ALLOW EVASION OF THE MOSQUITO IMMUNE SYSTEM

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The mosquito *Anopheles gambiae* L3-5 strain is capable of eliminating some lines of *Plasmodium falciparum* but not others. This elimination involves the mosquito immune complement-like system. A Quantitative Trait Loci (QTL) mapping was carried out to identify the *P. falciparum* gene(s) that allow some parasite strains to evade the *A. gambiae* immune system. The gene mapping was done in a *P. falciparum* cross between 2 lines that differ in their survival in *A. gambiae* L3-5; GB4 successfully infects the mosquito while 7G8 is mostly eliminated by melanotic encapsulation. Phenotyping of parental lines and progeny lines for survival/encapsulation in *A. gambiae* L3-5 presented only the two parental phenotypes. QTL analysis identified one main significant locus in chromosome 13 associated with the phenotype. Refined mapping of recombination sites in informative progeny lines narrowed down the locus to a region encompassing 41 genes. The QTL locus in chromosome 13 was confirmed independently by linkage group selection analysis of surviving oocysts from an infection of the mosquito using the non-cloned progeny of the GB4 x 7G8 cross. Analysis of ookinete gene expression of the QTL 41 genes identified 8 genes with at least 4 fold difference between GB4 and 7G8 lines. Sequencing the coding region of the QTL

41 genes identified 15 genes with non-synonymous polymorphisms between GB4 and 7G8 lines. Based on the expression differences and sequence polymorphisms, 5 candidate genes were selected for detailed genetic analysis by testing phenotype changes between survival and encapsulation, after allele replacement in *P. falciparum*. Identification of *P. falciparum* gene(s) that allow evasion of the mosquito immune system may be important to understand malaria transmission and could be a target for transmission blocking strategies.

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EVIDENCE OF RECOMBINATION IN THE X-CHROMOSOME CENTROMERIC REGION IN *ANOPHELES GAMBIAE* MOLECULAR FORMS FROM AN AREA OF PUTATIVE SECONDARY CONTACT

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Anopheles gambiae M and S molecular forms are typically strongly reproductively isolated and clearly identifiable based on a SNP in the multi-copy IGS rDNA region, co-segregating with the single-copy insertion of a SINE element located approximately 1 Mb apart from the X-centromeric IGS region. However, an area of putative secondary contact has been recently detected at the westernmost extreme of M and S range. Preliminary indications of discordant M and S genotypes at the two X-linked markers near the centromere in female samples suggests that introgression and inter-locus recombination may be occurring in this area. This hypothesis is intriguing because recombination is known to be highly reduced in centromeric regions, and this is believed to have played a significant role in the incipient speciation process ongoing within *A. gambiae*. Here we present data from M and S female (N=275) and male (N=392) samples collected in Safim village in Guinea Bissau. Notably, males provide the opportunity to separate recombination, as distinct from heterozygosity, along the hemizigous X-chromosome. Results from IGS and SINE PCR-genotyping show: i) a 22% frequency of SINE MS-heterozygotes in females (consistent with previous data) and an absence of heterozygotes in males (as expected for a single-copy X-linked marker); ii) a 34% and 9% frequency of IGS MS-heterozygous pattern in females and males, respectively, strongly supporting the occurrence of recombination within even the most centromere-proximal region of the X chromosome; iii) the occurrence of discordant SINE/IGS genotypes in 12% and 18% of SINE-M and SINE-S females, respectively, and in 10% of SINE-M and SINE-S males, showing that recombination is occurring in both molecular forms. Moreover, multilocus SNP analysis carried out on a subsample of males provides estimates of recombination along the whole X-chromosome and novel original insights on M and S form status in their putative secondary contact zone.

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GENOMES IN FLUX: 'REAL-TIME' DYNAMICS OF INCIPIENT SPECIATION IN *ANOPHELES GAMBIAE*

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Anopheles gambiae s.s. is considered to be in the process of incipient speciation into two molecular forms (M and S), originally defined by a single pericentromeric rDNA-IGS marker on the X chromosome. The molecular forms are sympatric throughout much of west and central Africa, but show broad bionomic differences which can extend malaria transmission in time and space. Genetic differentiation between M and S forms tends to be concentrated into genomic islands, which are resilient to gene flow. However, this genomic heterogeneity varies markedly between areas in line with levels of contemporary gene flow, most notably in Guinea-Bissau where the highest levels of inter-form gene flow are observed. Using Illumina Goldengate genotyping and whole genome resequencing we investigated the stability of M/S differentiation in Guinean samples collected in 1993 and in 2010. In the older samples genomewide differentiation, though very heterogeneous, clearly partitioned the molecular forms suggesting at least partial reproductive isolation. In the recent samples general M-S differentiation has decreased and a more complex within-population structure has emerged, with groupings exhibiting different degrees of resemblance to 'typical' M and S forms. Instability in population structure is not unexpected in an area of such high gene flow, but we also show adaptation-driven changes in genomic differentiation between the molecular forms from low gene flow areas. These data highlight that the dynamics of genome divergence in *Anopheles gambiae* speciation are occurring in real time, making the speciation process of relevance to contemporary control programmes.

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THE GENETIC BASIS OF HUMAN HOST CHOICE IN THE MALARIA VECTOR *ANOPHELES GAMBIAE*

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The predominant malaria vector *Anopheles gambiae s.s* preferentially takes it blood meals from human hosts, often at rates as high as 90% in natural populations. The adaptation of these mosquitoes to human hosts has a genetic basis in the olfaction system, which includes several key gene families - the odorant receptors (ORs), odorant binding proteins (OBPs) and ionotropic receptors (IRs). To identify *An. gambiae* genes responsible for human host preference, we conducted a quantitative trait loci (QTL) mapping experiment based on introgressive backcrosses between the anthropophilic *An. gambiae* and the zoophilic *An. quadriannulatus*, in which F1 females were backcrossed to *An. quadriannulatus* males. These backcross females were subjected to a host-choice experiment in an olfactometer in which they were presented with a human and cow odor. Only individuals that selected the same odor on three consecutive days were included in the experiment. A total of ~15,000 individual backcross females were run through host-choice experiments, resulting in two pools totaling 432 mosquitoes with divergent host preferences. We are using 24 microsatellite markers to genotype individuals from the two pools and performed QTL analysis using R/QTL. Preliminary results based on 13 markers identified one highly significant QTL that explains 16% of the phenotypic variance. This QTL region is estimated to span a 10 Mb region

and contains several ORs, OBPs and IRs. These genes are candidates for being involved in the adaptation of *An. gambiae* to its human host and were sequenced in six anopheline species to identify those that show evidence of positive selection.

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DENGUE 2 INFECTION ALTERS MICRORNA EXPRESSION IN *AEDES AEGYPTI*

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Emerging studies show that important avenues in the post-transcriptional regulation of gene expression occur via small RNA regulatory pathways. Non-coding RNAs (ncRNAs) are key features of these pathways, and critical molecules involved in their biogenesis and function are conserved in plants, insects and mammals. To identify products of anti-viral RNA interference in vector mosquitoes, deep sequencing small RNA (sRNA) libraries were prepared from DENV2-fed *Ae. aegypti* females (RexD strain) and matched un-infected controls at 2, 4, and 9 days post-infection (dpi). An earlier publication described DENV2-derived viral sRNAs (viRNAs) across three size classes: unusually small RNAs (usRNAs) (14-19nts), canonical siRNAs (20-24nts), and piRNAs (25-30nts) (Hess et al, BMC Microbiology, 2011). In the present analysis, these libraries were mined to determine whether substantive changes occur in mosquito microRNA (miRNA) levels during DENV2 infection. Reads were aligned to mIRBase release 17 hairpin database (mirbase.org). MiRNAs with over 50 reads across treatment groups and showing $\geq$ 2 fold-changes were chosen for further study. Our analysis reveals that significant changes to specific miRNA levels occur in DENV2-infected mosquitoes compared to un-infected controls. Moreover, some miRNAs showed coordinated enrichment or depletion at both 2 and 4 dpi, substantiating the hypothesis that they are important to the establishment of virus infection, whether by being exploited by the virus or as part of an anti-viral mechanism. Coordinately co-regulated miRNAs or *miRNAs include miR155, mIR2755, miR281 and miR277, among others. miRNAs were classified by type: conserved (homologous to previously reported miRNAs), *miRNA (complementary to miRNA), non-canonical or unclassified. Although the precise part played by each differentially expressed miRNA remains to be elucidated, orthologous miRNAs in other animals are important effectors of cellular differentiation, neurogenesis, transcriptional regulation, nutritional metabolism and the regulation of apoptosis. Our results show an intriguing new way in which major cellular processes of mosquitoes respond to arbovirus infection.

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GENETIC REGULATION OF VECTOR MOSQUITO SALIVARY GLAND DEVELOPMENT

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Understanding mosquito salivary gland development is critical given the importance of this tissue in blood feeding and pathogen transmission. Our recent survey of the mosquito genomes indicated that mosquitoes have orthologs of many genes that regulate embryonic salivary gland development in *Drosophila melanogaster,* a well-characterized insect genetic model organism. The expression patterns of a large subset of these genes were assessed during development of *Aedes aegypti,* an emerging model for vector mosquito development. These studies revealed that the early stages of *Ae. aegypti* salivary gland development significantly differ from that of *D. melanogaster.* We are now using an RNAi knockdown strategy to investigate the roles of genes expressed in the developing *Ae. aegypti* salivary gland. Functional characterization of *cyclic-AMP response element binding protein A (crebA)* indicates that this gene encodes a key regulator of secretory function in the *Ae. aegypti* salivary gland. These studies highlight the need for further analysis of mosquito developmental genetics and may foster comparative studies of salivary gland development in additional vector insect species.

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MEDUSA: A NOVEL GENE DRIVE SYSTEM FOR CONFINED SUPPRESSION OF MOSQUITO POPULATIONS

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Following successful field trials of sterile GM mosquitoes designed to control dengue fever, interest is now growing in the use of gene drive systems, such as X-shredders, capable of inducing a population crash as they spread. These systems hold much promise for wide-scale disease control; however issues arise from their potential to spread across international borders. We propose a novel gene drive system, Medusa, capable of inducing a local population crash without spreading into neighboring populations. Medusa consists of four components - two at a locus on the X chromosome and two at a locus on the Y chromosome. A maternally-expressed, X-linked toxin and a zygotically-expressed, Y-linked antidote suppress the female population because only males can protect themselves against the effects of the toxin. A zygotically-expressed, Y-linked toxin and a zygotically-expressed, X-linked antidote ensure that the two constructs are always inherited together. We use simple population genetic models to explore the dynamics of the Medusa system. An all-male release is preferred since males don't bite and, if released over two generations, Medusa is expected to induce a population crash within seven generations for modest release sizes. Re-invasion of wild mosquitoes can lead to the population eventually rebounding; however this can be prevented by small, regular releases of Medusa males. The Medusa system could serve as a proof of principle for invasive population suppression systems such as X-shredders. We describe molecular solutions to chromosomal anomalies that could interfere with Medusa dynamics.

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VACCINATION WITH EXCRETORY/SECRETORY PRODUCTS CONFERS PARTIAL PROTECTION IN A MURINE MODEL OF FILARIASIS

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Infection with filarial worms can cause severe and debilitating diseases in both humans and animals. While many vaccine candidates have been studied in filariasis, our understanding of protective immune responses in a permissive model to filariasis is incomplete. In this study, we evaluated preparations of worm antigens for protection against challenge infection in the BALB/c Litomosoides sigmodontis model. The fractions included LS, soluble antigens of a homogenate of adult worms, and ES, excretory/secretory products of adult female worms. 6-8 week old female BALB/c mice were given 3 intraperitoneal injections of 10 micrograms LS or ES with CpG/Alum and subsequently challenged with 40 infectious larvae subcutaneously. 8 weeks after infection mice were euthanized and parasite burdens were determined. Mice that were vaccinated with LS antigen showed no significant protection against challenge infection compared to control mice. Mice vaccinated with ES product, however, harbored 60% fewer adult worms than control mice. While mice vaccinated with LS and ES produced similar levels of IgG antibodies to both antigen preparations, analysis by western blot demonstrated that ES vaccinated mice recognized different ES proteins than LS-vaccinated mice. Currently, we are in the process of conducting mass spectroscopy to identify a ~160kda protein that was strongly preferentially recognized by ES-vaccinated mice. No substantially large differences were observed between the two vaccinated groups with regards to lymphocyte

proliferation or Th1 and Th2 cytokine production in response to ES or LS. However, IL-10 responses to parasite antigens were substantially decreased in ES vaccinated mice. Analysis of worm counts at early timepoints suggest that ES vaccination does not disrupt L3 migration through tissues. Work is underway testing whether ES vaccination blocks the ability of filarial worms to induce immune regulation.

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IMMUNO-PROPHYLACTIC EVALUATION OF RECOMBINANT CUTICULAR COLLAGEN (COL-4) AND ABUNDANT LARVAL TRANSCRIPT (ALT) USING MULTIVALENT STRATEGY IN EXPERIMENTAL FILARIASIS

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Human lymphatic filariasis is commonly known as elephantiasis and is a profoundly disfiguring disease caused by the parasitic nematodes such as *Wuchereria bancrofti*, *Brugia malayi* and *B. timori*. There are no vaccines to aid control programmes although the current estimate for people living in endemic areas are in the range of billions over 81 countries. Nematode parasites have a complex life cycle and hence vaccine development efforts may require incorporation of antigens representing various stages to improve efficacy. In this regard, we have evaluated the vaccine potential of cuticular collagen (COL-4) that forms a major component of the nematode cuticle. Further, we have also investigated the protective efficacy of COL-4 in combination with L3 stage specific antigen ALT-2. Accordingly, the col-4 gene was PCR amplified from infective stage (L3) stage cDNA library of *W bancrofti* and was cloned in prokaryotic expression vector. The 15kDa recombinant His-tagged protein was over expressed by salt inducible system and was purified by metal-affinity chromatography. The human immune response of the rWbCOL-4 was analyzed with various clinical sera and was found to show significant reactivity with endemic normal sera (putatively immune). The immunoprophylactic studies were carried out in *Meriones unguiculatus* (Jird) model by immunizing COL-4 either alone or in combination with ALT. The COL-4 immunized group gave 75 % protection and interestingly the combination groups (COL-4 +ALT) were found to be significantly higher in the range of 85%. Humoral and cellular immune responses suggest more of Th2 type response in parasite clearance. Hence, we report for the first time the role of COL-4 as a putative vaccine candidate in eliciting protective immune response in experimental filariasis.

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THE *ACANTHOCHEILONEMA VITEAE* PRODUCT ES-62 SUPPRESSES PATHOGENESIS IN COLLAGEN-INDUCED ARTHRITIS BY TARGETING OF THE IL-17-PRODUCING CELLULAR NETWORK AT MULTIPLE SITES

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ES-62 is an immunomodulatory, phosphorylcholine-containing glycoprotein secreted by the rodent filarial nematode *Acanthocheilonema viteae,* which has been found to be protective in the collagen-induced arthritis (CIA) mouse model of rheumatoid arthritis (RA). As IL-17 has been reported to play a pathological role in the development of RA, we investigated whether targeting of IL-17 may explain the protection afforded by ES-62 in this model. The CIA model employs DBA/1 mice, which progressively display arthritis following immunization with type-II collagen. The protective effects of ES-62 were assessed by measurement of cytokine levels, flow cytometric analysis of cell populations and *in situ* analysis of joint inflammation. ES-62 was found to down-regulate a number of different IL-17 responses in the CIA model. Firstly, it acts to inhibit priming and polarisation of IL-17 responses by targeting a complex IL-17-producing network, involving signalling between dendritic cells and/or $CD4^+$ T cells. Secondly, ES-62 directly targets Th17 cells by down-regulating expression of MyD88 to suppress responses mediated by IL-1 and TLR ligands. Further, ES-62 interferes with migration of T cells and this is reflected by direct suppression of CD44 up-regulation and, as evidenced by *in situ* analysis, dramatically reduced levels of IL-17-producing cells, including lymphocytes, infiltrating the joint. Finally, there is strong suppression of IL-17 production by cells resident in the joint, such as osteoclasts within the bone areas. Targeting of IL-17 responses by ES-62 presumably reflects the need of the parasite to dampen down host inflammatory responses that could be dangerous to parasite or even host. However, the multi-site manipulation of the initiation and effector phases of the IL-17 inflammatory network is notable and we believe further strengthens the argument for exploiting this potent helminth-derived immunomodulator, in the development of novel therapeutics for RA.

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LITOMOSOIDES SIGMODONTIS INFECTION INDUCES BASOPHIL SUPPRESSION IN RESPONSE TO ALLERGEN IN A MOUSE OVALBUMIN ALLERGY MODEL

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Helminth infections have been shown to protect against allergic disease, yet the mechanisms by which this occurs are not fully understood. We have begun to evaluate the effects chronic helminth infections have on the ability of effector cells of allergy to respond to allergen. Mice were sensitized to ovalbumin (OVA) and then infected with Litomosoides sigmodontis for 10 weeks. To assess basophil responsiveness, whole blood of sensitized mice was incubated with OVA and basophil activation was quantified by intracellular IL-4 expression. 20% of basophils from OVA-sensitized mice expressed IL-4 above basal levels, while basophils from OVA-sensitized mice that were chronically infected demonstrated no increase in IL-4 expression. Stimulation with anti-IgE antibody also showed basophil suppression in chronically infected mice, suggesting that decreased basophil responsiveness to OVA was due to a general suppression in IgE-mediated signaling. Infection was also associated with decreased proliferative responses of splenocytes to anti-CD3/anti-CD28 stimulation and decreased IL-4 and IL-5 production in response to OVA. These findings suggest helminth-mediated protection is due to both direct effects on allergy effector cells as well as inhibition of signals that drive allergic responses. Though we found no difference in IL-10 production in response to OVA, infected animals had increased IL-10 production in response to parasite antigen. Interestingly, the effect infection has on basophils may be rapid, as preliminary studies demonstrate that implantation of adult worms can suppress basophils in as little as 3 days. Ongoing experiments are investigating the effects infection has on mast cell function and clinical allergic disease.

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INTERIM SUCCESS IN THE DEVELOPMENT OF A *LOA LOA* AND MICROFILARIA-SPECIFIC ANTIGEN TEST

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Severe adverse events in patients with high levels of circulating microfilariae of *Loa loa* treated with either ivermectin or DEC have halted mass drug administration campaigns in areas of Africa endemic for these filariae. To identify individuals at risk for severe post-treatment sequelae, diagnostics that are *Loa*-specific and quantitative for microfilariae are urgently needed. To identify and quantify microfilariae-specific protein targets for a rapid immunoassay, we performed trypsin digestion followed

by mass spectrometry of *Loa loa* microfilarial excretory/secretory (E/S) product. Microfilariae from 4 patients with loiasis were collected following therapeutic apheresis, purified, and then incubated for 24-48 hours in serum free media. The E/S product was then concentrated and analyzed by nanobore reverse-phase liquid chromatography-tandem-MS (nanoRPLC-MS/MS) following trypsin digestion, after which the obtained spectra were searched against the recently completed putative proteome of *Loa loa* using SEQUEST. Of 15462 potential proteins in the proteome, 1274 (8.2%) E/S proteins of *Loa loa* microfilariae were identified. Twenty-five of the most highly abundant proteins are currently being explored as targets for a molecular-based diagnostic. Moreover, these E/S proteins were cross-referenced with a nanoRPLC-MS/MS analysis of proteins found specifically in the urine of patients with loiasis (and not in normal control urine). Four urinary proteins were identified that had no or little homology to human proteins; these are also being assessed for their diagnostic utility. Additionally, rabbits were injected with concentrated E/S and specific Ll-ES antisera raised. Purified IgG from this Ll E/S-specific antisera has been used to develop a sandwich ELISA that can detect Ll ES in human sera. Our data suggest that E/S products of Ll can be detected in human blood; studies examining the utility of such an antigen assay are currently underway in hopes of having a quantitative, microfilaria-specific circulating antigen test for use in blood and/or urine.

1009

DYNAMICS OF ANTIBODY RESPONSES BY ISOTYPES AND IGG SUBTYPES IN LABORATORY MODELS FOR ONCHOCERCIASIS

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Onchocerca volvulus, the causative agent of "river blindness," infects approximately 37 million people worldwide. Of these infections, 270,000 may result in blindness and 500,000 in vision impairment. The surveillance mechanisms for the detection of *O. volvulus* infections in humans is based on an IgG4 Elisa test using the recombinant antigen OV-16. This study examined the immune responses against OV-16 by IgM, IgG and the four IgG isotypes. Six chimpanzees were laboratory inoculated with 200-400 microfilria of *O. volvulus* microfilaria, and serum samples and skin snips were collected monthly for 4-5 years post inoculation. Negative sera samples were assayed and the cutoffs for positivity were determined by their mean plus 3 standard deviations. The Elisa values from IgG1, IgG2, IgG3, and whole IgM levels were not adequate indicators of infection, either due to low dynamic spectrum of OD values or responses that were detected later than IgG4 or whole IgG. The OD values for IgG4 were positive at 13.8 months post inoculation (median=15.5, range=7-18), approximately 1.2 months before the animals became microfilaria positive. Elisa values for whole IgG become positive at 11.4 months post-inoculation (median=13, range=4-15), which was 3.6 months before skin snips were detected positive. Our findings indicate that whole IgG reactivity may detect infections 2-months earlier when using the IgG4 OV-16 Elisa.

1010

PROTEOMIC PROFILING OF MICROFILARIA-EXPOSED HUMAN DENDRITIC CELLS IDENTIFIES HOST PATHWAYS ASSOCIATED WITH DENDRITIC CELL DYSFUNCTION

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Dysregulation of antigen presenting cells such as dendritic cells (DC) and macrophages (MΦ) is one of the many mechanisms proposed to mediate the profound filarial-specific T cell hyporesponsiveness seen in lymphatic filariasis. In fact, we have previously shown that live microfilariae (mf) of *Brugia malayi* induce apoptotic cell death in human monocyte-derive DC (mDC) and also modulate their ability to activate T effector cells. To characterize more completely heretofore unrecognized pathways and/or processes in mDCs influenced by mf exposure, the proteomes of mf-exposed and -unexposed mDCs were analysed by nanobore reverse-phase liquid chromatography-tandem-MS (nanoRPLC-MS/MS) following trypsin digestion. The obtained spectra were searched against *Homo sapiens, B. malayi* and *Wolbachia* databases using SEQUEST. A total of 1395 proteins were observed to be 2-fold regulated (spectral abundance) in mf-exposed mDCs that significantly correlated ($p<0.0001$) with transcriptional data analyzed independently using human microarray analysis. Interestingly, compared to mf-unexposed DC, mf upregulated (by ~4 fold) the cell surface and soluble forms of ICAM-1, processes mediated by IL-8, IL-17F and TNF-α– pathways. Ingenuity™ pathway analysis suggested that mf significantly downregulated ($p< 0.0001$) the mammalian target of rapamycin (mTOR), eukaryotic initiation factor (eIF) 2, eIF4 and p70S6K pathways as well as metabolic pathways involved in pyruvate metabolism and protein ubiquination. Interestingly, live mf secrete homologues of human FKBP1 (cyclophilin) a negative regulators of mTOR signaling. Because mTOR inhibitors (e.g. rapamycin) induce apoptosis in human DC and down-modulate the DC function required for T cell activation, functional studies on the mechanisms of mf-induced inhibition of mTOR are underway to provide the mechanistic link between the internalization of mf antigens and dysfunction seen in human DC induced by mf.

1011

TETRAVAX-DV, A LIVE ATTENUATED TETRAVALENT DENGUE VACCINE, IS SAFE, HIGHLY IMMUNOGENIC, AND INDUCES PROTECTION AGAINST A CHALLENGE DOSE OF VACCINE

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Dengue virus (DENV) has become the most important arbovirus worldwide with approximately 36 million cases of dengue fever and more than 2 million cases of severe dengue occurring annually. Several live attenuated tetravalent DENV candidate vaccines are being evaluated in clinical trials and the most advanced candidate has entered Phase 3 trial in numerous dengue-endemic countries. TetraVax-DV, a live attenuated dengue vaccine developed by the NIAID has been evaluated in several Phase I trials in the US and is poised to enter a Phase 2 trial in Brazil. In developing TetraVax-DV, we first evaluated the safety, replication kinetics, and immunogenicity of 8 different monovalent dengue vaccines in 15 separate trials to identify those candidates most suitable for inclusion in a tetravalent vaccine formulation. We then evaluated 5 different tetravalent admixtures to determine which combination would generate the most suitable safety

and immunogenicity profile. In that study, TetraVax-DV TV003 was very well tolerated by flavivirus-naïve adult subjects and elicited a trivalent or better antibody response in 90% of vaccinated subjects following a single subcutaneous dose. Because of these early promising results, we decided to further evaluate the safety and immunogenicity of TV003. In addition, we evaluated the ability of a single subcutaneous dose of TV003 to protect against challenge with a second dose of vaccine given 6 months after the first dose. 56 subjects were enrolled in this trial; 40 subjects received TV003 and 16 received placebo. Each of the components of TV003 was given at a dose of 1,000 PFU. Six months after receipt of the first dose of vaccine, subjects were challenged with a second dose of vaccine (or placebo). Subjects were followed in an identical manner after first and second doses. Viremia and safety labs were assessed on days 0, 3, 6, 8, 10, 12, 14, and 16 after each immunization. Specimens were collected for serological analysis on study days 0, 28, 56, 90, 150, and 180 post-immunization. Following the first immunization, the vaccine induced a tetravalent neutralizing antibody response in 71% of vaccinees and a trivalent or better response in 92% of vaccinees. Complete safety and immunogenicity data following the first vaccination will be discussed. The safety, absence of viremia, and immunologic response of vaccinees to the challenge dose of vaccine will also be discussed.

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IMMUNOGENICITY AND SAFETY OF A TETRAVALENT DENGUE VACCINE IN CHILDREN AND ADOLESCENTS IN LATIN AMERICA

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A tetravalent dengue vaccine (TDV) comprising recombinant, live, attenuated viruses, one per serotype (CYD-1-4), is being evaluated for protective efficacy in phase III. The objective of this study was to assess the safety and immunogenicity of TDV in endemic areas in Latin America. A randomized, controlled, observer-blind (first and second injections) and single blind (third injection) phase II clinical trial was conducted among healthy children aged 9 to 16 years in Colombia, Honduras, Mexico and Puerto Rico (ClinicalTrials.gov CT00993447). Participants received either 3 doses of TDV (n=401) or 2 doses of saline followed by a dose of Tdap (Adacel) vaccine (n=199) at Months 0, 6 and 12. Solicited injection site and systemic reactions were recorded daily for 7 and 14 days respectively after each injection. Plaque reduction neutralization test ($PRNT_{50}$) antibody titers against the TDV parental viruses were measured before and 28 days after each injection. The median age of participants was 12.6 and 52% were females. No vaccine-related serious adverse events were reported. Solicited injection site and systemic reactions after the first injection were reported by 31.6% and 57.9% of subjects in the TDV group and 27.6% and 54.3% of subjects in the Control group. A decrease in reactogenicity rates was observed after the second and third TDV doses. Most reactions were mild. Injection site pain and headache were most frequently reported. Seropositivity rates [antibody titers ≥10 (1/dil)] one month after the 3rd TDV vaccination, were: 94.2%, 98.9%, 100%, and 98.9% for serotypes 1-4, respectively. The corresponding geometric mean titers were 320, 486, 594, and 273. The percentage of seropositivity after 3 TDV doses was 98.6% for at least 3 serotypes, and 93.4% for all 4 serotypes. A three-dose TDV regimen had a satisfactory safety and reactogenicity profile and elicited neutralizing antibody responses against all four serotypes in children and adolescents in Latin America. These results are consistent with those from prior phase I and II clinical trials.

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IMPACT OF PRE-EXISTING IMMUNITY ON THE SAFETY AND IMMUNOGENICITY OF DENVAX, A TETRAVALENT DENGUE VACCINE

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An effective vaccine against dengue viruses (DENV) must be safe, immunogenic, and capable of eliciting a long-term protective immunity against all four serotypes. Pre-existing immunity to flaviviruses can significantly impact the safety and immunogenicity of a tetravalent dengue vaccine. Here the safety and immunogenicity of DENVax, a novel tetravalent dengue vaccine, was assessed in previously dengue-exposed AG129 mice (deficient in IFN type-I and II responses) and non-human primates (NHP). DENVax consists of a molecular clone of DENV-2 PDK53 and three chimeras, engineered to express the structural glycoproteins of DENV-1,-3, and -4. The AG129 mice and NHPs were pre-exposed (by intradermal or subcutaneous injection, respectively) to DENV-2 16681 or DENV-4 1086 and boosted by the same route with DENVax vaccine 42 and 60 days later, respectively. In both species, all animals infected with wt DENV-2 or wt DENV-4 mounted a strong neutralizing antibody response to the homologous virus and weak cross-reactive responses to the other DENV serotypes. In the presence of pre-existing immune responses to wt DENV-2 or wt DENV-4 there was no detectable viremia to any of the DENVax viruses following DENVax immunization. Analysis of the neutralizing antibody responses in individual animals following DENVax immunization showed that in both species the dominant neutralizing antibody specificity was against the DENV serotype to which pre-existing immunity had already been established. Interstingly pre-existing immunity to DENV-2 had a pronounced immunopotentiating effect on neutralizing antibody responses against all four serotypes. Overall, findings from this study suggest that pre-existing immunity has no detrimental effect on DENVax immunogenicity. Rather, neutralizing antibody responses to multiple DEN serotypes were enhanced. These preclinical studies support the safety and immunogenicity of DENVax administration in endemic countries; a Phase 2 clinical study directly addressing this issue is in progress.

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NEEDLE-FREE DELIVERY OF A CHIMERIC DENGUE-2 PDK-53-BASED TETRAVALENT VACCINE (DENVAX) IN NON-HUMAN PRIMATES

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To affect dengue vaccine delivery globally and in diverse clinical settings, an easy, practical and safe delivery method is required that enables the administration of vaccine in a needle-free fashion. Needle-free delivery approaches such as those using jet injectors are an attractive alternative to conventional needle and syringe injection. In this study we administered a tetravalent dengue vaccine, DENVax on day 0 and 60 by two routes of administration (subcutaneous; SC and intradermal; ID) using a novel,needle-free delivery device in non-human primates (NHP). Following SC priming with DENVax, the DENVax-2 component of the

tetravalent vaccine demonstrated a significant replication advantage over the other three chimeric DENVax components since only DENVax-2 RNA was detectable in the serum of vaccinated animals. In contrast, no viremia to any of the vaccine components was observed after ID administration. Analysis of the neutralizing antibody responses showed that vaccinated animals mounted primary and secondary neutralizing antibody responses to all four DENV serotypes. The DENVax-1,-2, and-3 vaccine components were the most immunogenic moieties of DENVax. In general, responses elicited by both routes were comparable but seroconversation rates following priming were superior via the SC route (100% for all four serotypes). In addition, the SC needle-free delivery of DENVax induced comparable neutralizing antibody responses to those induced by needle-and syringe. The protective efficacy of tetravalent DENVax was assessed against challenge with wild-type DENV-2. No viremia was observed in immune animals. In conclusion, the tetravalent DENVax vaccine administered with a needle-free vaccine delivery device has the potential to impact future mass vaccination campaigns by providing a safe, efficacious, and cost-effective dengue vaccine product targeted at the world market.

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PRECLINICAL TESTING OF A RECOMBINANT SUBUNIT VACCINE FOR DENGUE

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Dengue viruses are a major cause of morbidity and mortality throughout the tropics and subtropics. It is estimated that more than 120 countries currently have endemic dengue virus transmission and 55% of the world's population is at risk of infection. Globally, there are between 70-500 million infections each year, of which 2.1 million are clinically severe, resulting in more then 21,000 deaths annually. While a licensed dengue vaccine is not yet available, several vaccine candidates are currently being evaluated in clinical trials. Live attenuated vaccines for dengue have faced issues with interference between the four viral components. To overcome this issue Merck is evaluating a tetravalent recombinant subunit vaccine to protect individuals against dengue virus-induced disease. Preclinical studies have been conducted in non-human primates to evaluate the immunogenicity of tetravalent formulations prepared with different adjuvants and administered following different immunization schedules. The vaccine has been evaluated in both dengue naïve and experienced animals. These studies have shown the capacity of the recombinant proteins to induce durable, balanced tetravalent responses without evidence of interference. Efficacy as determined by protection from viremia following challenge with wild type viruses has also been demonstrated. Data from these preclinical non-human primate studies will be presented.

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THE DENGUE VACCINE INITIATIVE PROJECT IN COLOMBIA AND THAILAND: BURDEN OF DENGUE INFECTION IN CHILDREN AND ADULTS OF SANTA CRUZ COMUNA OF MEDELLIN AND BANG PHAE DISTRICT OF RATCHABURI PROVINCE

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Dengue infection is a major public health problem in both Colombia and Thailand, likely early adopters of dengue vaccines. Colombia experienced its largest epidemic with almost 157,000 DF cases and 217 deaths in 2010 and Thailand has reported provincial incidence rates up to 698/100,000 person years. In preparation for the upcoming dengue vaccine, the Dengue Vaccine Initiative is conducting epidemiological studies in Colombia and Thailand. The data from Ratchaburi province, Thailand and Medellin, Colombia will be used to estimate the burden of dengue infection in both adults and children, providing essential evidence for decision-making for vaccine introduction. DVI is conducting a passive facility-based surveillance complemented by a healthcare utilization survey (HUS) and a sero-survey to determine the burden of dengue in Bang Phae district of Ratchaburi province in Thailand and Santa Cruz comuna of Medellin in Colombia. In the surveillance, febrile patients between 1-55 years-of-age are evaluated for dengue infection. For the sero-survey, 2000 randomly selected residents are enrolled to estimate age-specific sero-conversion rate. From the HUS, we identify the proportion of febrile cases missed by the passive surveillance. Passive fever surveillances were launched in October and November, 2011 in Bang Phae Community Hospital and Santa Cruz Hospital, respectively. It has been a season of low caseload for both sites with only 173 and 77 subjects enrolled by March 2012, respectively. Thus far, we have 3 and 15 cases of acute dengue infection, including 3 primary and 12 secondary infections, examined using IgM ELISA in Bang Phae and Santa Cruz, respectively. In the sero-survey, 61% (n=1191) were found to be positive by IgG indirect ELISA among 1955 samples processed in Colombia. The first bleeding for the sero-survey in Thailand was just completed in mid-May, 2012. More data will be available for presentation by the end of 2012. The data generated, in addition to other economic, behavioral, market-demand collected in both sites, will be used to build comprehensive national investment cases of dengue vaccine in Thailand and Colombia. The investment cases will be used as models for other countries in the respective regions to facilitate accelerated development and introduction of safe and effective dengue vaccines.

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DENGUE VACCINE INITIATIVE: A MULTI-CENTER STUDY OF THE ECONOMIC BURDEN OF DENGUE INFECTION AND HOUSEHOLD WILLINGNESS TO PAY FOR DENGUE VACCINES

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As a dengue vaccine approach licensure, there is a need to examine the potential benefits of vaccine introduction. The Dengue Vaccine Initiative is conducting studies on dengue societal cost of illness (COI) and the private willingness to pay (WTP) for dengue vaccines in Ratchaburi province, Thailand; Medellin, Colombia; and Nha Trang, Vietnam. The data will be used to estimate the potential economic benefits of vaccination strategies, providing an improved evidence base for policymakers. The COI study estimates the direct/indirect costs associated with 200 dengue-confirmed cases at each site, both inpatient and outpatient. A survey instrument has been designed to collect a) the out-of-pocket costs for medicines, diagnostics, health service delivery, and transport and b) indirect cost due to productivity losses of patients and their care takers. Data will be collected during the first visit at treatment facilities, at 10-14 days post presentation, and at 28 days, if the patient is not fully recovered. The total COI per case will be calculated from the sum of out-of-pocket payments, indirect costs, and the facility treatment cost net of any patient co-payments. To estimate household demand and WTP for hypothetical vaccines against dengue infection, we administer a study questionnaire to 400 households at each site. Qualitative information regarding perceptions of dengue infection and the local need for dengue vaccines, and the perceived effectiveness of alternative prevention activities was collected during focus groups and pre-tests conducted in each site to refine the instrument. Respondents will be presented with one of five randomly assigned prices and asked how many vaccines they would purchase for the household and which family members would receive the vaccine. COI models will be developed to estimate the cost per dengue case based on severity and age. An economic private demand count model will estimate the average number of vaccines purchased per household as a function of vaccine price, efficacy, household perceptions of dengue severity and likelihood, as well as household socio-economic characteristics. Both parametric and non-parametric estimates of average WTP can provide information regarding the private benefits of vaccination. In parallel with epidemiological investigations conducted in the same areas, the package of results will provide an evidence for policy making for dengue vaccine introduction.

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INTENSIVE TRAINING IN MALARIA DIAGNOSIS TO STRENGTHEN THE MALARIA CONTROL PROGRAM IN ANGOLA AND MOZAMBIQUE

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Microscopic identification of *Plasmodium* spp. is the gold standard for the laboratory diagnosis of malaria. The identification of *Plasmodium* parasites to the species level can be determined by the examination of stained blood smears, but it relies on the technical expertise of the microscopist. Refresher training is essential to maintain and improve this expertise. We developed training tools and approaches to build training workshops in malaria diagnosis such as: 1-A digital training module with an archive of 800 digital images with different *Plasmodium* spp. presentations from clinical specimens obtained worldwide; 2- An archive of 150 microscope slides with thick and thin Giemsa-stained smears to enhance the practical exercises; 3- A training approach based on pre and post-tests comparison to verify the progress of participants. Each workshop consisted of examination of morphologic features of *P. falciparum*, *P. malariae*, *P. ovale* and *P. vivax* using digital images and stained slides in addition to the appropriate use of RDTs. The pre- and post- tests consisted of examination of 20 slides. Participants' scores were determined based on the percentage of slides that were identified correctly. We report the results obtained from intensive training in microscopy for diagnosis of malaria conducted in Angola and Mozambique between 2008 and 2011. To ascertain the short-term benefit of these trainings we analyzed pre- and post-test data from 120 laboratorians trained from 2008 and 2011. The data accumulated show that pre-test and post-test scores for Mozambique (N=61) were 50% and 80%, respectively. The results obtained in Angola were very similar (N=60) with the pre-test and post-test averages being 54% and 80%, respectively. We believe that intensive training on microscopic diagnosis of malaria should be a significant part of the malaria control programs in different countries.

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POSITIVE DEVIANCE: AN INNOVATIVE APPROACH TO IMPROVE MALARIA PREVENTION AND TREATMENT PRACTICES AMONG MOBILE AND MIGRANT WORKERS IN CAMBODIA

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Reaching mobile and migrant populations is one of the key strategies in the containment and elimination of artemisinin resistance in the Greater Mekong Subregion (GMS). Positive Deviance (PD) is an asset-based behaviour change approach with the underlying notion that every community has certain individuals (positive deviants or champions) whose malaria prevention and treatment practices result in better health outcomes than their neighbours. Malaria Consortium (MC) supported Cambodia's National Malaria Programme to pilot PD among residents and migrants in three villages in Sampov Loun district. The PD pilot aims to identify and promote good health seeking practices in both communities. The baseline survey conducted in Aug 2010 (n=309), suggested that knowledge about malaria and prevention were high in both communities but health-seeking behaviour for fever could be improved (residents 44.4%; migrant 33.3%), The PD process included 6 steps: pre-orientation meeting, community orientation, situation analysis, PD inquiry, participatory analysis, and community feedback. During the process, 13

in-depth interviews and 6 group discussions were conducted to identify the champions. For example, we identified a female migrant worker who never gets malaria by always sleeping under a bed net, wearing long sleeved clothes, covering her legs with a scarf while watching TV, and immediately going to the health centre when ill. All PD practices were shared with other community members through a 6 month PD-informed intervention which included training of volunteers, interactive health education sessions, role plays, art competitions and an advocacy seminar. An follow up survey is conducted in Mar 2012 (n=378) to better evaluate this intervention. Data entry and analysis is in progress and results will be presented in the ASTMH meeting, but preliminary results suggest that PD can serve as 1) a malaria intervention targeting migrants; 2) an alternative or supplementary method to deliver existing BCC/IEC interventions; and 3) an innovative model to promote community-based, bottom-up approaches.

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PARASITE-BASED MALARIA DIAGNOSIS: ARE HEALTH SYSTEMS IN UGANDA EQUIPPED TO IMPLEMENT THE POLICY?

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Effective coverage of parasite-based malaria diagnosis remains limited in malaria endemic countries. This study assessed the health systems capacity to absorb parasite-based malaria diagnosis (PMD) as an intervention at primary health care facilities in Uganda. In a cross sectional survey, using multi-stage cluster randomisation, level II (HCII) and III (HCIII) health facilities in 11 districts in Uganda were assessed for tools, skills, staff and infrastructure, and structures, systems and roles necessary for the implementing PMD. Health facilities: Out of the 125 heath centers (HC) evaluated, 64 (51%) were HCII and 49% were HC III. PMD was available at 30 (24%) of the HCs, microscopy in 18(30%) of the 61 HC IIIs the lowest level with laboratory facilities and RDTs in 12(20%) of all facilities surveyed. Three-months'-long stock-outs of oral and parental quinine were reported at 48% and 39% of the health facilities respectively. On average, half of the approved staff positions were vacant. All facilities had out-patient (OPD) registers, but did not uniformly capture vital mortality data. Health workers: Only 18%(131/730) of the recommended staff positions at the health centres were filled.. Out of the 131 health workers interviewed, 86 (66%) were nursing assistants. Only 47 (36%) of these health workers were sufficiently knowledgeable in managing severe malaria. Fifty six (43%) had received on-job training and supervision on malaria management within the 6 months prior to the survey. The top three reasons for referral were 1) poor response to treatment 66 (35%), 2) need for blood for transfusion 55(30%) 3) need intravenous fluids 31(17%). Overall, one-in-ten of the patients received adequate referral. Primary health care facilities had inadequate human and infrastructural capacity to effectively implement universal parasite-based malaria diagnosis. The priority capacity building needs were 1) training of health workers in fever management, 2) recruitment of qualified staff, 3) supply chain and stock management systems 4) referral and quality control systems.

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ANTIMALARIAL MARKETS AND TREATMENT-SEEKING BEHAVIOR FOR SUSPECTED MALARIA IN MYANMAR AND CAMBODIA: RESULTS FROM ANTIMALARIAL OUTLET AND HOUSEHOLD SURVEYS 2011-2012

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Artemisinin-resistant parasites were first detected around the Thai-Cambodia border, and there is evidence that resistance may be emerging in eastern Myanmar. Resistance to antimalarial drugs has historically spread from Southeast Asia to Africa, threatening recent global malaria control progress. Monitoring antimalarial markets and treatment-seeking behavior is key for informing and monitoring resistance containment strategies. The ACTwatch research program conducted nationally representative antimalarial outlet and household surveys in 2009 and 2011 in Cambodia. Data from these studies reflect changes in the antimalarial market and treatment-seeking behavior in the context of initiatives to improve case management coverage, such as the Village Malaria Worker program, as well as challenges to maintaining coverage due to stock-outs in the public and private sectors. Population Services International (PSI) conducted baseline household and private sector antimalarial outlet surveys in eastern Myanmar in 2011/2012. Results are representative of the private antimalarial market and treatment-seeking behavior in areas that comprise the Myanmar Artemisinin Resistance Containment (MARC) project, where partners are implementing resistance control strategies focused on replacing the widespread use of artemisinin monotherapy with ACT. Results from these studies and implications for improving coverage of appropriate case management and containing spread of artemisinin resistance will be discussed.

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AWARENESS ON THE AFFORDABLE MEDICINES FACILITY FOR MALARIA IN THE PRIVATE SECTOR IN GHANA

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The World Health Organization (WHO) estimates that about 40% of the world's population, mostly those in the Sub-Saharan Africa are at high risk of malaria. Malaria causes significant morbidity and mortality in Ghana. Artemisinin-based combination therapy is the recommended treatment for uncomplicated malaria but these medications are expensive making it inaccessible to people who need them. The Affordable Medicines Facility for Malaria (AMFm) is a mechanism to subsidize ACTs to increase access to Artemisinin-based Combination Therapy (ACTs) for treatment of malaria. To increase awareness of ACTS and acceptability of the initiative, an intensified Behavioural Change Communication and training of health workers was rolled out nationwide. The objective of this study is to determine the knowledge of private pharmaceutical outlets on the Affordable Medicines Facility for malaria (AMFm) and on the co-paid ACTs .One hundred and fifty one (151) pharmacies and five hundred and ninety one (591) LCS shops were randomly selected from the most urbanized and least urbanized districts across the entire country and monitored for three consecutive months. In-depth structured interview, using a mix of open-ended and closed questions, were used to determine health workers knowledge on the AMFm and the ACTs. Six months after commencement of the communication campaign on AMFm and ACTs, 95.4% respondents surveyed in the Licensed Chemical Shops and Pharmacies interviewed were aware of the AMFm initiative and the co-paid ACTs. The awareness level was higher in the urban areas (76%) than in the rural areas (33%) (p-value < 0.05). Awareness on the AMFm and on the co-paid ACTs

was higher in the Licensed Chemical Stores (98.5%) as compared to the Pharmacies (81%). Awareness of the AMFm has become high and knowledge of the co-paid ACTs has been increased due to the intensive campaign that were undertaken, thereby making demand for the ACTs with the green leaf logo very high in Ghana.

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MONITORING ANTIMALARIAL DRUG USE IN RELATION TO NATIONAL DRUG POLICY

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The majority of malaria-related deaths occur in sub-Saharan Africa and two of the most vulnerable groups identified are children under the age of five years and pregnant women. Historically, malaria has evolved resistance to the main first line antimalarial drugs, such as chloroquine and sulphadoxine-pyrimethamine. This has necessitated changes in national drug policies in African countries over the past decade towards artemisinin combination therapies (ACTs). The length of time between policy changes and their subsequent implementation, following the emergence of resistance, directly affects public health because of the mortality rates associated with a lower treatment efficacy. In this work, we have extracted data on treatment taken by children aged under five as a response to fever to estimate drug use and pressure on the African continent and describe the relationship with national policy changes. The work has been limited to chloroquine and sulphadoxine-pyrimethamine, as sufficient recent data on ACTs were unavailable. We interrogated databases from Demographic Health Surveys (DHS) and Multiple Indicator Cluster Survey (MICS) from 43 countries in Africa over the period 1993-2010. Based on the 99 studies that were included in our analysis over this period we have fitted logistic regression models, for chloroquine and sulphadoxine-pyrimethamine, for the proportion of fever cases treated with each drug over time (measured since policy change away from the drug), with country included as a random effect. We find that while drug use post policy change does exhibit a downward trend, there is still significant usage of these outdated drugs. We discuss the implications of drug use and its relationship to national policy for effective care of malaria, and for managing resistance.

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SURVEILLANCE IN THE SOUTH PACIFIC, THE ESTABLISHMENT OF BASELINE DATA AND MONITORING FOR CHANGE IN MALARIA DRUG RESISTANCE PROFILES

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Vanuatu and the Solomon Islands (SI) are progressing towards malaria elimination. Artemether-lumefantrine (Coartem™), an artemisinin combination therapy (ACT), was introduced as first line therapy for both *falciparum* and *vivax* malaria in these countries in 2008, and forms the cornerstone of the malaria control/elimination programs in both countries. Reports of artemisinin resistance developing in SE Asia demonstrate that ongoing surveillance is essential to detect and monitor for artemisinin resistance. Additionally, these countries are among the few where ACT is used for treatment of both *falciparum* and *vivax* malaria allowing for detection of potential changes in resistance profiles of both species without CQ and SP pressure. Our aim is to obtain base-line data during Coartem introduction allowing for future detection of changes in drug resistance profiles. Samples collected from baseline epidemiological surveys in Tafea Province, Vanuatu and Temotu Province, SI along with samples from a Coartem efficacy study conducted in Malaita Province, SI were investigated for the prevalence, distribution and origins of drug resistant *Plasmodium* parasites by examining sequence polymorphisms within known drug resistant markers (*pfcrt, pfdhfr, pfdhps, pvdhfr* and *pvdhps). Pfcrt* analysis revealed 100% (Tafea and Malaita) and 98% (Temotu) of parasites carried the K76T mutation indicative of CQR; microsatellites flanking *pfcrt* are similar to those in Papua New Guinea suggesting these CQR parasites share a common ancestry. Variations in *pfcrt* genotypes were detected between and within these two countries, even on smaller islands with limited populations. In Vanuatu three *pvdhfr* alleles were observed with the majority containing the double polymorphism, 58R/117T. Similarly, *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. Unlike the variability exhibited in the *pvdhfr* gene, 100% of samples possessed the drug sensitive *pvdhps* allele 382S/383A/512K/553A/585V.

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DEVELOPMENT OF GENOMICS RESOURCES FOR *BABESIA MICROTI*, AN EMERGING INFECTIOUS DISEASE AGENT IN THE UNITED STATES

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Babesia microti is the principal cause of human babesiosis and one of the most common transfusion-transmitted pathogens in the United States. The parasite has a worldwide distribution and has been cited as an emerging health threat in the United States. *B. microti* is primarily transmitted to humans by the tick vector, *Ixodes scapularis*, but transmission also occurs perinatally and through blood transfusion. We have sequenced and annotated the genome of a clinical isolate of *B. microti*. Comparative genomic studies with other protozoa reveal that *B. microti* has the smallest nuclear genome among all apicomplexa with three chromosomes encoding ~3,500 polypeptides, including species-specific genes and gene family expansions. Genome-wide reconstruction of functional networks revealed the minimal metabolic requirements for intraerythrocytic parasitism by protozoan parasites. Furthermore, unlike all other Apicomplexa, its mitochondrial genome is circular. This study resulted in the identification of several targets suitable for diagnosis and treatment of human babesiosis. Remarkably, genome-wide phylogenetic analyses indicate that *B. microti* is significantly distant from all species of Babesidae and Theileridae and defines a new clade in the phylum Apicomplexa. Efforts are now underway to sequence the genome and transcriptome of six new isolates of *B. microti*. These studies will inform on *B. microti* pathogenesis, genetic diversity, evolution and virulence and will open new avenues for future design of improved diagnostic and treatment strategies.

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AGE-DEPENDENT GENETIC ASSOCIATIONS WITH *CRYPTOSPORIDIUM* INFECTION IN BANGLADESHI CHILDREN

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Infection by *Cryptosporidium*, a protozoan parasite, is one of the major causes of diarrhea in children globally. Up to 12% of diarrheal disease in children less than 2 years of age in developing countries may be attributed to *Cryptosporidium,* with more severe outcomes in malnourished children. Infection early in childhood can lead to stunting as well as impaired cognitive development. The human immune response to *Cryptosporidium* has not been well-characterized, especially in young children where the burden is the highest. Prior studies demonstrated the association of HLA Class I and II alleles with cryptosporidiosis in young children, suggesting a genetic involvement in the host response to *Cryptosporidium* infection. To identify host genetic factors that may play a role in susceptibility to infection, we conducted a genome-wide association study (GWAS) in 374 children from Dhaka, Bangladesh participating in a birth cohort using 6.6 million single nucleotide polymorphisms imputed to 1000 Genomes. Diarrheal stool samples were collected less than 24 hours after a report of a new diarrheal episode and tested for the presence of *Cryptosporidium*. Of these 374 children, 58 had cryptosporidiosis within the first year of life. For the 350 children with 2 years of follow-up, 99 had at least one *Cryptosporidium* infection. Associations were calculated across the human genome using an additive genetic model. Comparing children with or without infection within the first year of life, two regions reached a genome-wide significance: 1q32 ($p=1.5x10^{-7}$) and 11q24 ($p=4.6x10^{-7}$). An additional suggestive signal was found in chromosomal region 5p15 with a p-value of $8.7x10^{-7}$. We extended the association to compare children with or without infection in the first two years of life and two regions showed genome-wide significance: 2p21 ($p=3.5x10^{-7}$) and 4p14 ($p=3.7x10^{-7}$). This study suggests that host genetic factors may be important for susceptibility to *Cryptosporidium* infection in early life and these host genes may be age-dependent.

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BREAST MILK IGA AGAINST *CRYPTOSPORIDIUM* PROTECTS BANGLADESHI INFANTS FROM INFECTION

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Cryptosporidium diarrhea is a major contributor to morbidity among infants in the developing world. There are limited therapeutic options for young children, and there is currently no vaccine available. Here we investigated whether *Cryptosporidium*-specific breast milk IgA could protect infants from *Cryptosporidium* infection. A longitudinal cohort study of 226 infants was followed by twice-weekly home visits from birth through the first year of life in a Dhaka slum community. Diarrheal and non-diarrheal monthly surveillance stools were collected from each infant and tested for *Cryptosporidium* using qPCR. Breast milk samples were collected from each mother in the first month after birth and tested for IgA against *Cryptosporidium* oocysts. By 12 months of age, 40% of children had been infected with *Cryptosporidium*. Infants whose mothers had high levels of anti-Cryptosporidium breast milk IgA had a lower risk of *Cryptosporidium* infection (defined as a diarrheal or monthly stool sample positive by PCR for Cryptosporidium, p=0.021) and had a higher chance of survival free of *Cryptosporidium* infection (p = 0.039). This study is the first to report that breast milk antibodies can be protective against *Cryptosporidium* infection. This is a significant finding, as immunity against this parasite has been thought to be primarily cell-mediated. Our findings suggest that passive immunity may have important implications for prevention of *Cryptosporidium* infection in breastfed infants as well as for treatment of fulminant disease in immunocompromised adults.

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DISTRIBUTION OF DRUG RESISTANCE AND ITS GENETIC BASIS IN GLOBAL ISOLATES OF *TRICHOMONAS VAGINALIS*

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The emergence of antibiotic-resistant pathogens remains one of the most challenging problems in health care. Antibiotics influence microbial communities in the human body by changing microbial ecology, with the potential to increase resistance. *Trichomonas vaginalis* is the most prevalent non-viral sexually transmitted pathogen in the world. Although trichomoniasis had long been regarded as a sexually transmitted infection of minor importance, evidence implicates *T. vaginalis* as a contributor to a variety of adverse outcomes such as increased transmission of HIV, cervical cancer and adverse pregnancy outcomes. The parasite is commonly treated with the nitroimidazole group of antiprotozoal agents but ~5% of isolates display drug resistance. Analysis of global genetic variability in *T. vaginalis* isolates indicates that resistant isolates cluster within one of two primary subpopulations, suggesting that it will be possible to isolate genes and pathways that are involved in drug resistance. In order to address the prevalence of drug resistance phenotype in different geographical regions, we have performed resistance assays with two of the most commonly used drugs (metronidazole and tinidazole) in 187 *T. vaginalis* isolates from seven geographical regions to: (1) confirm their drug susceptibility status and (2) determine if the resistance is heritable. Our analysis of clonally identical genotypes based on 21 *T. vaginalis* microsatellite loci in each geographical region did not identify more than three identical clones, suggesting the necessity for larger genetic screens. We are also developing genome wide screens to identify the genetic loci responsible for drug resistance, which will allow us to test if the same genes are responsible for drug resistance in different geographical regions. Comparison of *T. vaginalis* natural isolates exhibiting a range of drug susceptibilities is an important step towards understanding how the parasite modifies its molecular makeup with permanent genetic changes to overcome the challenge of drug pressure.

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IMPROVING THERAPEUTICS FOR THE TREATMENT OF CRYPTOSPORIDIOSIS USING HIGH THROUGHPUT METHODS

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Cryptosporidium parvum and *Cryptosporidium hominis*, the most common etiological agents of cryptosporidiosis, are the leading cause of waterborne diarrheal outbreaks in the United States and second most common cause (after rotavirus) of pediatric infectious diarrhea in Africa and Asia. Susceptibility to the parasite has been correlated with nutritional status in children, and chronic infection has been associated with decreased functional status. Infection is often self-limited in healthy individuals, but results in chronic, fulminant, and often fatal illness in immunocompromised populations. Nitazoxanide, the current therapeutic gold standard, is effective at reducing the duration of infection in immunocompetent individuals, but is no more efficacious than placebo in immunocompromised populations. The high morbidity and mortality in pediatric and AIDS patients, combined with unavailability of highly

active antiretroviral therapy makes cryptosporidiosis a severe public health problem in the developing world. While excessive cost makes the *de novo* development of anti-cryptospordial medications nearly impossible, drug repurposing may provide a more feasible solution. The discovery of new uses for already approved compounds with demonstrated safety records facilitates the efficient entry of candidate medications into clinical efficacy trials. We have developed a robust (Z' score= 0.27-0.41) cell-based high throughput screening (HTS) platform to test inhibitors of *Cryptosporidium parvum* and conduct follow-up testing. We have successfully screened the NIH Clinical Collection, a compound library of 727 approved drugs and experimental drug-like compounds, and identified 11 compounds with ≥ 90% inhibition, and 25 compounds exhibiting ≥ 80% inhibition *in vitro*. 20 compounds were identified as potential leads and repurchased for follow-up testing including the determination of IC_{50} and TD_{50}, potential for synergy, and to determine whether the drugs employ static or cidal mechanisms of parasite killing in order to inform prioritization for further *in vivo* follow up.

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WHOLE-GENOME CAPTURE OF *THEILERIA PARVA*, AN APICOMPLEXAN PARASITE OF CATTLE IN SUB-SAHARAN AFRICA

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East Coast fever (ECF), which occurs in eastern, southern and central Africa, is an acute fatal disease of cattle caused by the tick-transmitted intracellular apicomplexan pathogen *Theileria parva.* Every year ECF kills >1million cattle, the primary source of food and income for many smallholder African farmers. The "infection-and-treatment" immunization method (ITM) recently adopted in areas of sub-Saharan Africa induces long-term immunity based on CD8+ T-cell responses, but has significant logistical and economic drawbacks, making the development of an effective recombinant vaccine a high priority. In the last few years reverse vaccinology has emerged as a primary approach to identify putative vaccine antigens from genome sequence data. However, the application of reverse vaccinology to eukaryotic pathogens still presents several fundamental challenges. In the case of *T. parva*, those challenges start with the isolation of parasite DNA for genome sequencing. *T. parva* piroplasm DNA can be obtained from bovine blood in sufficient quantity for genome sequencing but results in the sacrifice of the bovine host. Here we report the successful capture and sequence of *T. parva* genomic DNA from a *T. parva*-infected lymphocyte cell line. The capture probe set was designed to cover 97% of the 8 Mb genome of the *T. parva* Muguga strain, including 98% of its annotated genes. The fragments captured from both Illumina and 454 libraries built from infected lymphocyte DNA mapped to 97-98% of the *T. parva* Muguga genome. Unmapped reads totaled less than 25% of the captured reads, establishing the selective preference of *T. parva* DNA over that of the host. Ongoing experiments will determine the success of this approach to capture *T. parva* genomic DNA from lymphocyte cell lines infected with divergent isolates of this species. These results demonstrate the feasibility of whole-genome sequence capture to isolate parasite DNA from a mix of parasite and host DNA. Furthermore, the results are directly applicable to a variety of human intracellular pathogens of similar genome size and complexity, including several apicomplexan parasites.

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THE BURDEN OF CRYPTOSPORIDIOSIS AND ITS EFFECT ON GROWTH IN A BIRTH COHORT OF CHILDREN IN AN URBAN SLUM OF SOUTH INDIA

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Cryptosporidiosis is a major cause of diarrheal disease in developing countries, predominantly affecting children under 5 years of age. Cryptosporidiosis in early childhood is reported to affect growth and development. To estimate the burden of cryptosporidiosis and its effect on growth preliminary data from an ongoing birth cohort study in an urban slum of South India were analyzed. Children (N=413) were followed biweekly from birth to 18 months of age to assess diarrhea and other morbidities. Stool samples were collected every 2 weeks and during a diarrheal episode (N=17,875) and tested for *Cryptosporidium* spp. by PCR. Anthropometric (height and weight) measurements were obtained monthly. Acute (wasting, WHZ < - 2 SD) and chronic (stunting, HAZ < - 2 SD) malnutrition at 18 months of age was assessed using the WHO Child Growth Standards 2006. The effect of symptomatic and asymptomatic cryptosporidiosis on stunting and wasting was assessed using a logistic regression model adjusted for crowding (>5 members/household), presence of older siblings, exclusive breastfeeding up to 6 months, low birth weight (<2500 grams, LBW), gender, religion, and socioeconomic status (SES). By 18 months, 190 (46%) children experienced one or more episodes of cryptosporidiosis. In 121 (63.8%) children who had only asymptomatic infection/s and 69 (36.2%), children who had at least one symptomatic infection the proportion with repeated episodes were similar (20 (16.6%) and 16 (23.2%), p=0.25). The mean (95% CI) ages at first asymptomatic and symptomatic infections were also similar (8.4 (7.2-9.6) vs. 8.8 (8.2-9.4) months, p=0.54). At 18 months, 122 (30%) children had stunting and 54 (13%) had wasting. Both symptomatic (OR: 1.9 (1.1-3.5), p=0.03) and asymptomatic (OR: 1.7 (1.1 -3), p =0.02) infections had a significant effect on stunting. LBW (OR = 3.5 (2 -6.3, p <0.001) and SES (OR =1.6 (1- 2.8), p= 0.04) were predictors for stunting. In children with LBW, symptomatic infection was predictive for stunting (OR= 3.9 (1 -15), p=0.04). Acute malnutrition was more pronounced in boys (p=0.01). These findings indicate that cryptosporidiosis was significantly associated with chronic malnutrition by 18 months of age.

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DIFFERENTIAL ANTI-GLYCAN ANTIBODY RESPONSES IN *SCHISTOSOMA MANSONI*-INFECTED CHILDREN AND ADULTS STUDIED BY SHOTGUN GLYCAN MICROARRAY

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Schistosomiasis is a chronic and potentially deadly parasitic disease that affects millions of people in (sub)tropical areas. Infected individuals do acquire immunity to *Schistosoma*, but this takes many years of exposure, multiple infections and treatments, and maturation of the immune system. Therefore, children are more susceptible to re-infection than older children and adults. This age-dependent immunity or susceptibility to re-infection after treatment has been shown to be based on antibody as well as T cell responses. Most antibodies generated during *Schistosoma* infection are directed against the abundant glycans expressed by the parasite, of which an unidentified subset appears to be protective. The structure of most of the glycan epitopes recognized by antibodies is however unknown. The interaction of antibodies with glycans can be studied efficiently and quantitatively using glycan microarray approaches in which small amounts of a large number of glycans are presented on a

glass slide. We have generated a shotgun glycan microarray containing natural N-glycan and lipid glycan fractions derived from 4 different life stages of *S. mansoni* and applied this array to the analysis of IgG and IgM serum antibodies in a selection of sera from children and adults living in an endemic area. For the first time, we have analyzed the anti-glycan antibody responses in *Schistosoma* infection against many different glycan elements simultaneously. The antibody responses against many of the *Schistosoma* derived N-glycan and lipid glycan fractions are on average higher in children than in adults and are dominated by IgM and may reflect differences in age or differences in length of exposure or infection. We have shown that the shotgun glycan microarray approach has strong potency to study antibody response profiles and allows the definition of patient groups as well as glycan element clusters to which antibody responses are generated and can be applied to select potential glycan vaccine elements.

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T REGULATORY LYMPHOCYTES AND IMMUNE RESPONSES TO SCHISTOSOME ANTIGENIC PREPARATIONS BY INDIVIDUALS WITH EITHER *SCHISTOSOMA MANSONI* "RECENT RE-INFECTIONS" OR "CHRONIC" INFECTIONS

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Patients chronically infected with *Schistosoma mansoni* exhibit high levels of circulating regulatory T cells (Treg), which are associated with immune regulation in murine experimental schistosomiasis. The functional role of Treg in human schistosomiasis has not yet been established, but it is hypothesized that they act to reduce proliferation and cytokine production by schistosome specific responder T lymphocytes upon appropriate encounters with schistosome antigens. We find that men with infections of 2 years or longer ("chronic") have higher levels of circulating CD4+/CD25hi Treg cells than men re-infected 8 months or less prior to being studied ("recent re-infections"). We have also phenotyped lymphocytes from these subjects using monoclonal antibodies against FoxP3, CD127, CD45RA, CD45RO, CD357 (GITR), HLA-DR, and CTLA4 and find that CD25hi and CD45RAneg parameters best characterize the FoxP3+ Treg population we are studying. Using a BrdU ELISA assay to measure Peripheral Blood Mononuclear Cell (PBMC) proliferation to Soluble Egg Antigens (SEA) or Soluble Worm Antigenic Preparation (SWAP), we see highly variable responses by individuals and by the two groups. Men with "chronic" infections with low PBMC responses to SWAP have increased responsiveness upon removal of their CD25hi/Treg populations using anti-CD25 magnetic beads, indicating that Treg play a regulatory role in their immune response to SWAP. We also find that two populations of men with "chronic" infections with different exposure histories have distinct Treg profiles. Men working as sand harvesters, most of whom were exposed to schistosomiasis from 2-4 years of age and were likely born of *S. mansoni*-infected mothers, have higher levels of circulating CD4+/CD25hi Treg cells than car washers, who may have only been exposed to infection in their adult years. These data provide new immunological insights into immunoregulation during human schistosomiasis and suggest possible further differences in the immunology of schistosomiasis infections based on their duration and exposure histories.

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A ROBUST AND REPRODUCIBLE PROCESS FOR PRODUCTION OF A SCHISTOSOMIASIS VACCINE

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Schistosomiasis causes significant morbidity and mortality in the developing world with recent studies indicating that the geographic extent and burden of the disease is higher than the official estimates. Although, Schistosomiasis is a treatable infection, the current treatments of choice do not provide an optimal strategy for controlling the disease. The high rate of post-treatment reinfection has made obvious the need for new approaches, such as vaccination, to complement the existing treatment initiatives. The increase in information regarding the mechanisms of immunity for Schistosomiasis infection has indicated that surface antigens may be effective vaccine candidates. Of this handful of proteins, some have already been shown to have potential as recombinant vaccines against Schistosomiasis at a pre-clinical level. In particular, the tetraspanin family of integral membrane proteins, highly abundant in the parasite tegument, has been shown to correlate with protective immunity in a mouse vaccine model, suggesting that this family of membrane proteins offers promise as a Schistosomiasis vaccine. The Pichia codon optimized DNA sequence of the extracellular domain of Sm-TSP-2 was synthesized and cloned into the Pichia expression vector pPinkα-HC. Here we describe the process development that led to the GMP manufacturing of one of the lead candidate antigens Sm-TSP-2 at a 20 L scale fermentation, and its formulation for phase I clinical trials. Throughout the process we confirmed the yield of recovery, the purity, and the integrity of the recombinant protein, as well as a comprehensive biophysical characterization of the protein itself.

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TARGETING SCHISTOSOME CERCARIAL PROTEASE FOR VACCINE DEVELOPMENT

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Parasitic blood flukes of the genus *Schistosoma* cause schistosomiasis, the most important helminthic infection in terms of morbidity and mortality in developing countries. Potential resistance to praziquantel, the only drug currently used to treat the disease, has already raised serious concerns about the necessity to discover and develop new drugs against schistosomes. The development of a subunit vaccine for schistosomiasis would also be a key step for disease control. Schistosome larvae (cercariae) are able to directly penetrate host skin facilitated by secretion of proteases from acetabular cells of cercariae in response to skin lipids. Recent proteomic analyses of secretions identified an S1A serine peptidase termed cercarial elastase (aKa or SmCE cercarial protease) as one of the most abundant proteins released by *Schistosoma mansoni.* SmCE is involved in both skin invasion and immune evasion. Preliminary work has shown that skin invasion by cercarie could be inhibited by serine protease inhibtors. To validate SmCE as a vaccine candidate, a proof of principle study will use ecotin (*Escherichia coli* serine protease inhibitor) as a model for protease directed antibodies. To apply this inhibitor to *ex vivo* skin, before exposure to cercariae, we are using a nanopatch technology with microneedles ensuring the delivery of ecotin to the epidermal layer of skin. In parallel, we are carrying out a second proof of principle study based on antibody-based inhibitors. We will "pan" SmCE against a diversity antibody library composed of biased Fab fragments designed to inhibit serine proteases.

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IMMUNO-MODULATION OF HUMAN IMMUNE RESPONSES DURING SCHISTOSOME INFECTION AND CONSEQUENCES OF HELMINTH TREATMENT PROGRAMS

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We have been involved in studies evaluating the need, safety and efficacy of treatment of paediatric schistosome infection. The results of these studies were recently published in a World Health Organisation document where we and others recommended that schistosome control programmes be extended to include pre-school children. The overall short- and long-term consequences of this recommendation need to be evaluated in light of experimental and field studies suggesting that helminth infection modulates immune responses directed against unrelated antigens. The factors affecting the magnitude and dynamics of the modulation of these immune responses in helminth-exposed people are still poorly understood. Thus, alongside the paediatric schistosome studies, we have been characterising the relationship between infection with the helminth *Schistosoma haematobium* and immune responses to unrelated antigens, including common allergens (house dust mite), self-antigens (nuclear antigens) and antigen from other pathogens *(Plasmodium falciparum* vaccine candidates). These immune responses have potentially important consequences for clinical allergy, autoimmune disease and malaria vaccine efficacy. We have also been conducting mechanistic studies involved in helminth-related immuno-modulation through comparative human studies. The studies, all conducted in the same two populations have provided significant insight into factors affecting host immune responses to different antigens during helminth infection and the effects of antihelminthic treatment on these responses across a wide age range of people with different histories of schistosome infection.

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REPEATED *SCHISTOSOMA JAPONICUM* INFECTIONS IN A SUBSET OF INDIVIDUALS FOLLOWING TREATMENT

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It has been shown that, at any point in time, a few individuals may be responsible for disproportionate transmission of infectious pathogens. However, for disease systems where reinfection is possible, are superspreaders the same individuals over time? To answer this question, we examined the incidence of repeated *Schistosoma japonicum* infections in two longitudinal cohorts in Sichuan, China. In each cohort, participants were tested for infection and treated with praziquantel at baseline, then followed prospectively and tested for infection two more times over a period of six (Cohort 1) and three (Cohort 2) years. Water contact behaviors were assessed and *S. japonicum* infections were promptly treated with praziquantel. *S. japonicum* infection prevalence at baseline was 47% (mean infection intensity 46 EPG) and 11% (mean infection intensity 2.6 EPG) in cohorts 1 and 2, respectively. The incidence of two consecutive infections was 1.5 times higher than expected in cohort 1 and 5.8 times higher than expected in cohort 2 ($p<0.001$ in both cohorts). As it is possible that individuals who are repeatedly infected with *S. japonicum* are the most highly exposed individuals in the population, we additionally predicted the expected incidence of repeated infection accounting potential exposure to cercariae-contaminated water sources using a data-adaptive, machine learning algorithm. The incidence of repeated infections was 1.3 ($p=0.013$) and 2.1 ($p<0.001$) times greater than expected in cohorts 1 and 2, respectively, accounting for host exposure. The clustering of infections within a limited number of hosts suggests infected individuals may be appropriate targets for intervention and surveillance. Moreover, fact that the repeated observation of schistosomiasis in the same individuals cannot fully be explained by host exposure suggests some individuals may be more susceptible to *S. japonicum* infection than their peers or, alternatively, these individuals do not, in fact have new infections, but have residual, uncured infections that persist despite treatment.

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ISOLATED AND PERSISTENT INTESTINAL SCHISTOSOMIASIS FOCI IN WESTERN PROVINCE OF BURKINA FASO

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The national schistosomiasis and soil transmitted helminthes control program in Burkina Faso implements mass drug treatment for underserved communities at risk for schistosomiasis since 2004. Urinary schistosomiaisis is endemic in all the districts of the country but intestinal schistosomiasis is restricted to the Dafra district in the Western province. We reviewed data collected in the village of Panamasso in the Dafra district from 2008 to 2012. The village is located in a humid area with high level of rainfalls favoring and sustaining ponds and small perennial water bodies. There is a tributary of the river *''We"* that spans through the village and constitutes the main source of water for domestic use for the 1427 inhabitants. The district has the perfect ecology, climate and freshwater to harbor snails of Biomphalaria genius, the intermediate host for *Schistosoma mansoni*. The communities depend on the water from the *''We"* river for various daily usage. Fishing is a major occupation sometimes creating itinerant groups that move and settle following fishing zones along the river. Risk factors of *S. mansoni* infection also include the total absence of even traditional latrines in the village and defecation in open area along the river and stagnant bodies of water within the village. The prevalence of *S. mansoni* was 30% at the onset of the program and increased yearly since 2008 reaching 38.54% in 2012 despite satisfactory PZQ treatment coverage of over 80%. Heavy intensity of infection has progressed from 8 % in 2008 to 37% in 2012 for people with > 400 eggs/gram. This review shows that in settings where the prevalence remains high, Schistosomiasis treatment frequency may need to be augmented to efficiently deal with *S. mansoni* transmission.

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ISOLATION OF *SALMONELLA SP.* FROM A *PTEROPUS GIGANTEUS* FRUIT BAT IN BANGLADESH

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Typhoid fever causes approximately 21 million cases and 0.2 million deaths worldwide annually with the highest incidence in South-East Asia. The etiologic agent of Typhoid Fever, Salmonella Typhi, is considered a host-adapted pathogen of humans. However, researchers identified that fruit bats (*Pteropus rufus*) were carriers of Salmonella Typhi in Madagascar in 1973. Based on this study in Madagascar, we conducted a cross-sectional study in three ongoing Nipah surveillance areas in Bangladesh to sample native fruit bats (*Pteropus giganteus*) for the presence of Salmonella Typhi and other Salmonella serotypes. From February to June 2010 we captured 302 Pteropus fruit bats, collected rectal swabs and placed them in Cary Blair media before transporting them to the Clinical Microbiology laboratory of icddr,b within 48 hours. Laboratory technicians used MacConkey agar, S-S agar and Selenite broth for isolation and S-S agar for sub-culture. We sent the isolate to the Enteric Diseases Laboratory Branch, CDC for serotyping. No Salmonella Typhi was recovered from any of the sampled bats. We recovered a single isolate of Salmonella of Group C1 from one juvenile female *P. giganteus* bat (prevalence 0.33%, 95% confidence interval 0.008%, 1.8%) from Faridpur district. The Enteric

Diseases Laboratory Branch, CDC identified this as Salmonella Virchow. Though we did not find any evidence of Salmonella Typhi, this does not exclude the possibility that Salmonella Typhi could be carried at least occasionally by *Pteropus* bats in Bangladesh. Salmonella serotype Virchow has not been previously reported from *Pteropus* bats. Salmonella Virchow had been isolated from humans in Bangladesh from two clinics. This bat may have become infected with Salmonella by drinking surface water contaminated with human feces. Since Salmonella serotype Virchow and other serotypes can be carried by bats, Pteropus bats may be involved in chains of salmonella infection that ultimately affect humans.

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EVALUATION OF TUBEX® TF, A RAPID DIAGNOSTIC TEST FOR TYPHOID FEVER, IN THE MIDST OF A LARGE, URBAN TYPHOID FEVER OUTBREAK - HARARE, ZIMBABWE 2011-2012

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Salmonella enterica serovar Typhi (*S.* Typhi) causes an estimated 22 million cases of typhoid fever and 216,000 deaths worldwide annually. We assessed the value of a rapid diagnostic test compared with blood culture during a large outbreak of typhoid fever in Harare, Zimbabwe. Suspected cases of typhoid fever were defined as persons who lived in or visited Harare since October 1, 2011, with ≥3 days of fever and one or more of the following symptoms: malaise, headache, vomiting, diarrhea, constipation, or cough. As of March 21, 2012, 3,932 suspected cases, including 1,857 hospitalizations and 2 deaths were reported. Fifty-two cases were confirmed by blood or stool culture. Cases ranged in age from 1-88 years (median 16 years); 54% were female. Cases were predominantly from the high-density suburbs of Kuwadzana (1,824) and Dzivaresekwa (919). The pulsed field gel electrophoresis pattern for seven of eight isolates was indistinguishable from that of two isolates from an outbreak in Zimbabwe in 2009 and from isolates from Malawi and Tanzania. We evaluated a rapid serum IgM antibody diagnostic test for typhoid fever, TUBEX® TF, compared to standard blood culture. Patients were enrolled at two facilities: a primary care polyclinic in the high-density Kuwadzana suburb of Harare and Beatrice Road Infectious Disease Hospital which provides inpatient care across Harare. Patients included in the analysis were ≥1 year old, residents of Zimbabwe, had a fever ≥38°C at time of enrollment and at least one of the symptoms noted above. As of April 2012, *S.* Typhi was recovered from 7 of 103 patients by blood culture; 70 cultures were negative and 26 were pending. To date, 13 serum samples, including 6 from culture-confirmed patients, were positive; 32 were negative, 31 were pending and 27 were excluded because of gross hemolysis. Sample collection is ongoing. In this large outbreak, preliminary results from TUBEX® TF testing correlated with blood culture results suggesting it might be a useful adjunct for clinical management and surveillance.

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IDENTIFICATION OF *IN VIVO*-INDUCED BACTERIAL PROTEINS DURING HUMAN INFECTION WITH *SALMONELLA ENTERICA* SEROTYPE PARATYPHI A

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We applied an immunoscreening technique, *In Vivo*-Induced Antigen Technology (IVIAT), to identify immunogenic bacterial proteins expressed in humans bacteremic with *Salmonella enterica* serotype Paratyphi A, the cause of paratyphoid fever. We were able to assign a functional classification to 15 of 20 proteins identified by IVIAT. Of these 15, the majority represent proteins with known or potential roles in the pathogenesis of *S. enterica*. These include proteins implicated in fimbrial structure, antimicrobial resistance, bacterial motility, heavy metal transport, bacterial adhesion, sugar transport, and anaerobic respiration. The five remaining antigens represent proteins with unknown functions. Transcripts for 15 of the 20 genes were previously identified in the blood of humans bacteremic with *S.* Paratyphi A or *S.* Typhi. We confirmed increased expression of mRNAs expressed by genes identified by IVIAT by quantitative-PCR. Of the 20 identified antigens, we examined differential immunoreactivities in acute versus convalescent phase human serum samples for five antigens; these five included BcfA, YcfL, StcD, Gp19, and one antigen of unknown function encoded by SPA0489. BcfA is a fimbrial subunit involved in bacterial adhesion with no homolog in *E. coli*. YcfL is a putative periplasmic lipoprotein. StcD is a putative exported protein. Gp19 is a lysozyme that facilitates biosynthesis of the cell wall during cell division. SPA0489 encodes a protein of unknown function that is unique to *S.* Paratyphi A. Since *S.* Paratyphi A is a human restricted pathogen, there are limited data on host-pathogen interactions. Additionally, there is currently no commercially available vaccine for *S.* Paratyphi A and diagnostic assays lack sensitivity and specificity. *S.* Paratyphi A antigens identified by IVIAT warrant further evaluation for their contributions to pathogenesis, and may have diagnostic, therapeutic, or preventive uses.

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SMALL SCALE POULTRY FARMING AND ZOONOTIC TRANSMISSION OF ANTIBIOTIC RESISTANCE IN RURAL ECUADOR

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Poultry farming is promoted for international development because poultry are inexpensive, an efficient source of protein, and have few associated cultural beliefs. While poultry farming offers a promising economic strategy, there is a risk of development of antibiotic resistant bacteria. Previous studies have reported shared resistant strains between humans and poultry workers in industrialized settings, but few studies have occurred in community settings. In developing countries the potential for zoonotic transmission may be elevated due to inadequate water, sanitation, and hygiene conditions. We collected fecal samples from humans (n= 741), chickens (n=294) and environmental media (n=530) in communities with active small-scale poultry farming operations in rural Ecuador between June 2011-February 2012. Most villages had backyard farms with coops close to households, and one village had a central larger facility. We took advantage of a natural experiment that occurred during the study when the latter village converted to backyard operations between sampling periods. Environmental samples were collected from

domestic (household drinking water and cooking/eating surfaces), outdoor (soil outside households), and coop (soil outside coop and surfaces) locations. We isolated E.coli from all samples and assessed phenotypic resistance to a suite of 12 antibiotics. Isolates were considered multidrug resistant (MDR) if they were resistant to more than 5 antibiotics. We observed high rates of MDR (67-88%) and resistance to fluoroquinolones (enrofloxacin and ciprofloxacin; 50-62%) in coop samples, rates comparable to industrial farming operations. In the village with a collective farming operation, MDR in human samples was significantly elevated during the period when the village had backyard farms (94% of samples MDR) as compared to the when they had a communal facility (28% of samples MDR; p<0.0001). MDR and fluoroquinolone resistant E.coli were also isolated from the domestic environment at higher rates during the time of backyard farming. Small-scale poultry operations are associated with high levels of resistant bacteria in both environmental and human samples in this community setting, especially for farms close to households. These results suggest that it would be safer to promote poultry farming in centralized facilities rather than in backyard operations.

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ANTIBIOTIC RESISTANCE VARIATIONS OF *ENTEROBACTER* SPP. WHICH ISOLATED FROM CYSTITIS OF CHILDREN IN IRAQ

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A total of 57(3.9%) *Enterobacter* spp. were isolated from 1474 urine specimens collected from children (1-7 years) with cysititis, during January to September 2010 at AL-Hakeem hospital in Najaf governorate, Iraq .The results showed that *Enterobacter* spp. were more isolated from females (68.4%) than males (13.6%). The highest month of isolation were recorded during February (6.6%) followed by July (5.8%). The antibiotic resistances of *Enterobacter* spp. isolates showed strongly resistant to Cephaloxin and Cephatoxime (100, 71.4)% and (80, 57.1)% in January and February respectively, while In March the isolates shows a strong resistance to Ceftraiaxone, Cephaloxin and Gentamicin (100%),while in April all isolates show 57.1% resistance to Cephatoxime and 42.8% for Nalidixic Acid while Ceftraiaxone, Nalidixic Acid and Ciprofloxacin were the drug of choice for treatment of *Enterobacter* spp. infections. In July the isolates were resist to Amikacin and Ciprofloxacin in low percentage (0%,11%) respectively. In August and September *Enterobacter* spp. shows a high resistance to Cephaloxin (83.3%,100%) respectively. The antibiotic resistances of *Enterobacter* spp. isolated from cystitis of children were related to seasonal variation in Iraq.

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CAMPYLOBACTER JEJUNI FLUOROQUINOLONE RESISTANCE TESTING BY DETECTION OF THE THR86ILE *GYRA* MUTATION USING REAL-TIME PCR

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Campylobacter jejuni is a major cause of human enteritis, but its treatment has been complicated by the worldwide emergence of resistance to fluroquinolone (FQ) antibiotics, such as ciprofloxacin (CP). FQ resistance is often conferred by Thr86Ile mutation in the *gyrA* gene and may be detected through molecular methods, but few such methods have been evaluated in the developing world. Our objective was to develop and validate a real-time polymerase chain reaction (RT-PCR) Thr86Ile *gyrA* mutation detection assay to detect *C. jejuni* FQ resistance. From 2006 to 2010, *C. jejuni* stool isolates were obtained from three hospitals in Lima, Peru (Hospital Del Niño, Materno Infantil and Emergencias Pediatricas). DNA was extracted by QIAamp DNA Mini Kit. Degenerated primers (773F 5'-CATCTTCCCTAGTCAAGCCT-3' and 773R 5'-AAGATATGGCTCTAGCAAGAC-3') were used to amplify *glyA* fragments and detect a 773-bp PCR product consistent with *C. jejuni*. Isolates confirmed as *C. jejuni* by PCR then had CP susceptibility determined by E-test. Thr86Ile *gyrA* mutation detection was performed by RT-PCR. TaqMan probes (HEX 5'-CCCACATGGAGATACAGCAGTTTATG-3'-BHQ2 and 6-FAM 5'CCCACATGGAGATATAGCAGTTTATG-3'BHQ1), and primers (CjFW gyrA 5'-TGCTGTTATAGGTCGTTA-3' and CjRV *gyrA* 5'-CCTTGTCCTGTAATACTTG-3') were designed by AlleleID-7 software, with the resultant PCR product analysed by Rotor Gene Q. Sensitivity and specificity of the Thr86Ile *gyrA* resistance mutation detection assay was determined using E-test as a gold standard. 189 isolates were confirmed as *C. jejuni*. 74.6% (141/189) of isolates were CP-resistant by E-test. The Thr86Ile *gyrA* mutation was detected in 100% (141/141) of CP-resistant and 0% (0/48) of CP-sensitive isolates. The Thr86Ile *gyrA* mutation detection assay showed a sensitivity and specificity of 100% by comparison to E-test. The RT-PCR *gyrA* Thr86Ile mutation detection assay was highly sensitive and specific in the detection of FQ resistant *C. jejuni*.

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OUTBREAK OF MULTI-DRUG RESISTANT *SALMONELLA* TYPHI, LUSAKA, ZAMBIA, 2011-2012

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Salmonella enterica serovar Typhi causes approximately 22 million typhoid fever infections worldwide each year and outbreaks are increasing across southern Africa as urban population growth overwhelms aging water and sanitation systems. In December 2011, clusters of suspected typhoid cases were identified at two city hospitals in Lusaka, Zambia. From December 2011 - February 2012, 416 suspected cases were reported through hospital-based surveillance. We conducted a retrospective review of laboratory records and a case-control study using hospital-identified cases and matched controls from a highly affected neighborhood reporting an attack rate of 59.1 cases per 100,000 per month to identify risk factors for typhoid fever. A suspected case was defined as a hospitalized person having fever ≥3 days and one or more of the following: abdominal pain, vomiting, diarrhea, constipation, headache, joint pain, muscle pain, malaise, negative malaria parasite test or lack of improvement with antimalarial medication from the affected-neighborhood. Laboratory record review revealed 145 *Salmonella* Typhi isolates recovered from January 2011 - February 2012. Among isolates tested locally for antimicrobial sensitivity, 7/71 (9.9%) were resistant to ciprofloxacin, 9/27 (33.3%) resistant to nalidixic acid, 66/108 (61.1%) resistant to chloramphenicol, and 34/112 (30.4%) resistant to cefotaxime. Of 248 neighborhood households tested, only 20% demonstrated residual chlorination in their drinking water at a level of at least 0.2mg/L. Neighborhood public drinking water sources tested negative for residual chlorine, and one sample was positive for *Escherichia coli*, an indicator of fecal contamination. Recommended control measures included emergency point-of-use water treatment interventions and community education about sanitation and hygiene until long term repairs in water and sanitation systems are made. Antibiotic resistance indicates the need to re-assess treatment recommendations and strengthen laboratory surveillance.

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NOT ALL DEFINED DAILY DOSES ARE EQUIVALENT: EXAMINING THE EFFECTS OF VARIABLE ANTIBIOTIC TREATMENT UPTAKE AND DURATION OF USE ON POPULATION ANTIBIOTIC RESISTANCE

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Antibiotic resistance costs more than $16 billion annually in the United States. Measuring population level antibiotic use is important because it is linked to emergence and prevalence of antibiotic resistance. The WHO advocates a measure called the Defined Daily Dose (DDD) to ascertain the point prevalence of antibiotic treatment in a hospital or community. While this measure takes into account the prevalence of treatment at a given point in time, it does not take into account the rates of treatment uptake and treatment cessation. We use transmission models to assess how variability in starting and stopping antibiotic treatment affects transmission of and competition between sensitive and resistant strains. A given defined daily dose (point prevalence of treatment) can result in a range of possible disease prevalences determined by how quickly people are starting and stopping antibiotic treatment. The faster people are starting and stopping, the lower the transmission strength of sensitive strains. When there are co-circulating antibiotic resistant and sensitive strains, reduced sensitive strain transmission allows resistant strain transmission to increase. Summarizing population level antibiotic use as a defined daily dose provides an incomplete picture of the effect that population level antibiotic use has. Just as the prevalence of disease can be approximated by the incidence of disease multiplied by the duration of disease, so can the prevalence of antibiotic use. Thus combined with the prevalence of treatment, measuring either the rate of uptake or the average duration of treatment would provide a greater understanding of the effects antibiotics have on population level transmission and resistance prevalence.

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ACUTE DIARRHEA IN INDIGENOUS ADULT POPULATION IN NEPAL

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Diarrheal disease is generally accepted as a major cause of childhood morbidity and mortality in developing countries. As population in developing countries have early and repeatedly exposed to enteric pathogens, acute diarrhea in adults is believed to be uncommon or relatively mild and has less public health concerns. Therefore, data on adult diarrhea etiology is scarcely available. We conducted a hospital-based surveillance in an indigenous adult population in Nepal. A stool specimen and demographic and clinical information was collected. Stool culture and identification of enteric bacteria by standard microbiology was conducted in Nepal. Confirmation of isolates and further testing was performed at AFRIMS. Six hundred cases with acute diarrhea and 600 non-diarrhea controls were enrolled. Mean age of cases was 36.5 years, 47% were male, duration of diarrhea before hospital visits was 29 hours and 11% had received prior medication. Ninety four percent of cases reported watery diarrhea and 12% reported blood in stool. Abdominal pain was commonly reported in 80% of cases while only 10% reported fever. The most common organisms detected significantly more frequently in cases than controls were mainly bacteria, *V.cholera* (22% vs 1%), ETEC (10% vs 2%), *Aeromonas* (9% vs 4%) and *Shigella* (8% vs 1%). Enteric viruses, rotavirus, adenovirus or norovirus were also found in 1-4% of cases and rarely in controls. Asymptomatic infections with *Giardia*, Enteroaggregative *E.coli* and Enteropathogenic *E.coli* were also common in Nepalese adults with approximately 9% each. *In vitro* resistance against fluoroquinolone was detected in *Campylobacter* (89%), *Shigella* (15%) and ETEC (9%). In conclusion, bacterial gastroenteritis is common in adults in Nepal suggesting immunity from previous exposure is of short duration or provides incomplete protection. Frequent watery diarrhea and severe abdominal pain were main reasons for adults with acute diarrhea to seek hospital care. Asymptomatic infections with organism frequently colonizing children were also surprisingly common in adults.

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LONG TERM IMMUNE RESPONSES TO THE COLONIZATION FACTOR OF *VIBRIO CHOLERAE* O1, THE TOXIN-COREGULATED PILUS ANTIGEN TCPA, ARE LOW IN MALNOURISHED PATIENTS WITH CHOLERA

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A key adhesion antigen of *Vibrio cholerae* O1 is the toxin co-regulated pilus (TCP) a polymer of TcpA. Following natural cholera, TcpA-specific mucosal and systemic antibodies are induced. A relationship between micronutrient deficiency and development of symptomatic cholera has also been previously observed. We wanted to determine if immune responses including lipopolysaccharide (LPS) and TcpA differ between malnourished and well nourished patients with cholera followed over a year. We included 54 patients with cholera and samples on days 2, 7, 30, 90, 180, 270 and 360 post-onset. Plasma was assayed for antigen specific responses. We assessed nutritional status by determining the "Body mass index" (BMI) and "Mid upper arm circumference" (MUAC) and reference values of the NCHS were used. Patients were grouped as malnourished ('MNour') and well nourished ('WNour').The median age of the study group was 27 years and 35% were females. 89% had severe dehydration in both the groups. The average intake of fluid among 'MNour' group was 4L and 'WNour' group was 8L (p=<0.001) as the same pattern was seen in the ORS intake (p=0.01).Utilizing BMI to stratify patients, IgA responses to TcpA were significantly lower at time points in the 'MNour' group (p=0.006-0.08). The LPS specific IgA antibody responses were also lower in this group (p=0.01-0.04) only from day 180 onwards. When classified by MUAC, IgA responses to TcpA were also lower in the 'MNour' group at day 2 (p=0.05), and from day 90 onwards (p=0.009 to 0.005). The LPS IgA responses were lower among malnourished cases followed up from day 90 to 360 (p=0.002-0.08).No differences were seen between 'MNour' and 'WNour' groups in IgG responses. In summary we show that malnourished patients with cholera have significantly lower IgA immune responses to TcpA and LPS. The relationship of this response with other indicators of protection including the memory B and T cell responses as well as responses in young children need to be evaluated with implications for vaccine intervention in high risk nutritionally deprived populations.

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UNDERNUTRITION BY A REGIONAL BASIC DIET ALTERS SMALL INTESTINAL MORPHOLOGY, BARRIER FUNCTION AND CELL-CELL JUNCTION GENE EXPRESSION IN WEANLING MICE

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Undernutrition is major risk factor for child morbidity and mortality in developing countries, with gut manifestations that include perturbed intestinal epithelial homeostasis, mucosal atrophy, and increased susceptibility to enteric pathogens. The purpose of our study was to evaluate changes in small intestinal morphology, barrier function and

gene expression in mice subjected to malnutrition by a regional basic diet (RBD; 10% protein, 2% fat, 88% carbohydrate). 3 to 4 week old male albino mice were provided an *ad lib* standard chow diet (15% protein, 20% fat, 65% carbohydrate) or the RBD for seven days. On day 7, we assessed weight gain, tail length, and intestinal permeability by the urinary lactulose/mannitol test. Following sacrifice, the ileum was harvested to measure crypt-villous dimensions (height, depth, area), as well as tight junction (claudin-2, occludin, ZO-1) and adherens junction (βcatenin) gene expression by RT-PCR. We found significant decreases in weight (23.8 ± 1.2 g vs. 17.4 ± 0.7 g, p<0.001) and tail length (8.2 ± 0.1 cm vs 7.2 ± 0.1 cm, p<0.01) in mice fed the RBD. Morphometric analysis of ileal villi and crypts revealed reductions in villous area (33940 μm^2 ± 2109 vs 27920 ± 760 μm^2, p<0.01) and crypt depth (160.0 ± 3.4μm vs. 119.5 ± 2.7 μm. p<0.05), but not villus heigh The ratio of urinary lactulose:mannitol was significantly increased in malnourished animals, indicating a greater degree of barrier dysfunction (0.68 ± 0.03 vs. 1.22 ± 0.04 p<0.001). RBD-fed mice showed down-regulation of claudin-2 and ZO-1. The regional basic diet induces failure to thrive in young mice. Our results show important associations between undernutrition, small intestinal morphology, gut barrier function, and alterations in tight junction and adherens junctions gene expression that require further study to elucidate links between nutrition, gut homeostasis, growth, and enteric infections.

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DEVELOPMENT OF A *SHIGELLA* VACCINE BASED ON GENERALIZED MODULES FOR MEMBRANE ANTIGENS

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Shigella is one of the most frequent causes of diarrhea in young children and infants in developing countries. In natural infection the immune response to the O antigen is protective but serotype-specific and *Shigella* comprises 50 different serotypes. No vaccine is available. Novartis Vaccines Institute for Global Health's not-for-profit mission is to develop effective and affordable vaccines for impoverished communities. Gram-negative bacteria naturally shed particles that consist of outer membrane lipids and outer membrane proteins in their native orientation and of soluble periplasmic components. These particles have been proposed for use as vaccines but the yield has been problematic. We developed a high yielding production process of genetically derived outer membrane particles we named Generalized Modules for Membrane Antigens (GMMA, also known as outer membrane vesicles). Yields of approximately 100 milligrams of membrane-associated proteins per liter were obtained from high density cultures of genetically modified *S. sonnei* in a 5 L fermenter supporting the feasibility of scaling up this approach as an affordable manufacturing process. Proteomic analysis of the purified particles showed the preparation to primarily contain predicted outer membrane and periplasmic proteins. Furthermore, we demonstrated the feasibility of using this process with other genetic manipulations, e.g. to reduce LPS endotoxicity and to modify immunogenicity by removing the immunodominant O antigen. GMMA were shown to confer protection against *Shigella* using the murine model of pulmonary infection. Intranasal immunization with GMMA with and without O antigen derived from *S. flexneri* 2a resulted in statistically significant survival after homologous challenge compared to placebo. Importantly, GMMA without O antigen derived from *S. sonnei* also protected against challenge with *S. flexneri* 2a, demonstrating the potential for heterologous protection. In conclusion, this work provides the basis for a large scale manufacturing process of GMMA for production of vaccines from Gram-negative bacteria and for the development of GMMA as vaccine candidate against *Shigella*.

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CHOLERA OUTBREAK IN TOFA AND SAMAWA WARDS - ZAMFARA STATE, NIGERIA; 2011

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Cholera remains a major public health problem in Nigeria usually occurring as large scale outbreaks leading to high morbidity and mortality. Between June-July, 2011; we investigated suspected cholera outbreak in Tofa and Samawa wards in Bungudu Local Government of Zamfara State, northern Nigeria to confirm the outbreak and implement targeted interventions. We conducted cross sectional study. A suspected cholera case was defined as any resident of Tofa or Samawa ward with at least three episodes of acute watery diarrhea with or without vomiting between 30th May and 4th July, 2011. We reviewed hospital records and used case-based line listing to collect patients' data. Environmental assessment was conducted. We collected and analyzed 5 stool and 2 water samples using Thiosulfate Citrate Bile Salts Sucrose and Mac Conkey agars. Data were analyzed using Epi info and Microsoft Excel. Altogether, 111 cases were recorded (attack rate: 0.59%) with 5 deaths (Case fatality rate: 4.5%). Females accounted for 50.5% (56) and males 49.5% (55) of cases (p= 0.9), age range was 4 months to 65 years, median age was 5 years. About half of cases (47.7%) were children between 0-4 years (p = 0.01), age-specific attack rate for 0-4 years age group = 1.06%. All (100%) stool specimens yielded V. cholerae 01, serotype Ogawa. Environmental assessment revealed unsanitary conditions and inadequate and unsafe water supply. Water samples tested negative for V. cholerae but yielded growths of *E. coli*. Bungudu LGA had confirmed cholera outbreak. We strengthened case management and conducted health education focusing on personal hygiene and environmental sanitation. Advocacy visit was paid to local authorities to intensify health education and provide adequate and potable water to affected communities.

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GLOBAL AND REGIONAL TRENDS IN CONTINUED FEEDING OF CHILDREN LESS THAN FIVE YEARS OLD WITH DIARRHEA: ANALYSIS OF DEMOGRAPHIC AND HEALTH SURVEY DATA FROM 1993 TO 2010

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Continued feeding is a critical component of successful home management of diarrhea in children, and has been associated with decreased mortality and incidence of diarrhea. Repeated episodes of diarrhea are associated with malnutrition in children, and feeding a child during and after diarrhea episodes is crucial in preventing adverse nutritional effects. Understanding region-specific trends in continued feeding practices for children with diarrhea would allow appropriate direction of efforts to prevent deaths in the regions where diarrhea-associated mortality is greatest. We examined global and regional trends in continued feeding during diarrhea episodes among children less than 5 years old using Demographic and Health Survey (DHS) data from DH conducted between 1993 and 2010 in Asia, Latin America, and Sub-Saharan Africa. Continued feeding prevalence estimates were weighted by each country's population of children less than 5 years old for each year. We performed linear regression to estimate the change per year (β in prevalence of continued feeding during diarrhea globally and by region. The population of children less than 5 years of age represented by the 89 DHS in this analysis was 589 million. Prevalence of continued feeding for each region as estimated from recent DHS surveys (2008- 2010) ranged from 33.3-49.8% in Latin America, 33.7-61.1% in Sub -Saharan Africa,

and 35.9-71.1 % in Asia. Between 1993 and 2010, globally, continued feeding for children with diarrhea appears to be unchanged (β=0.15, p=0.82). Likewise, no significant improvements in continued feeding were observed in Latin America (β= -0.10, p=0.78) or Sub-Saharan Africa (β= -0.15, p=0.27). Prevalence of continued feeding in Asia appears to be increasing significantly at 0.25% increase rate per year (p=0.05). Sub-Saharan Africa has the highest diarrhea-associated mortality of any region in the world but has seen no improvements in continued feeding for children with diarrhea since 1993.These findings indicate the need for quantitative and qualitative research to understand barriers to continued feeding on the part of caregivers, and renewed efforts to promote continued feeding as a core component of diarrhea case management in settings where the burden of diarrhea is high.

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TRENDS IN ANTIBIOTIC RESISTANCE AND DIARRHEAL DISEASE EPIDEMIOLOGY IN A MILITARY POPULATION IN THE PERUVIAN AMAZON, 2003-2011

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In Peru, where antibiotic use is unregulated, the effective treatment of diarrheal disease is often complicated by the development of antibiotic resistant organisms. We aimed to investigate the trends in diarrheal disease etiology and antibiotic resistance in a military population in the Peruvian Amazon in order to guide diarrhea treatment. From 2003 to 2011, diarrheal disease surveillance was conducted among personnel at the Vargas-Guerra Army Base in Iquitos, Peru. All individuals experiencing diarrhea were requested to present to the army health post where a stool sample was taken for culture. Diarrheagenic bacteria were isolated from 34.5% of the 638 cases. From the 215 samples in which a single bacterial pathogen was isolated, *Shigella flexneri*, *Enterotoxigenic E. coli* (ETEC) and *Enteroinvasive E. coli* (EIEC), were the most common pathogens and were identified in 47.0%, 30.2%, and 5.6% of samples, respectively. There were no trends in the prevalence of *Shigella flexneri* or EIEC over the study period, however, the prevalence of ETEC decreased significantly (Odds Ratio= 0.86, 95% CI=0.75, 0.97; p=0.02). Of 101 isolates of *Shigella flexneri* cultured, 95.0% demonstrated resistance to tetracycline, 89.1% to chloramphenicol, 84.2% to ampicillin, and 80.2% to cotrimoxazole. Resistance of *Shigella flexneri* to ciprofloxacin and azithromycin remained low (0% and 8.9%, respectively). There were no significant trends in resistance to any other antibiotics over time. These data demonstrate a high prevalence of *Shigella flexneri* and diarrheagenic *E. coli* among diarrhea cases in a military population in the Peruvian Amazon. Although antibiotic resistance to penicillins and sulfa antibiotics remains high in this population, more appropriate or less frequent use of certain antibiotics may have led to decreasing resistance.

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CLUSTER OF GUILLAIN-BARRÉ SYNDROME DUE TO A WATERBORNE OUTBREAK OF *CAMPYLOBACTER JEJUNI* INFECTION -- SAN LUIS RIO COLORADO, SONORA, MEXICO AND YUMA, ARIZONA, UNITED STATES, 2011

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From May 31-June 16, 2011, a cluster of 15 suspected cases of Guillain-Barré Syndrome (GBS) which sometimes follows *Campylobacter jejuni* infection, was reported in San Luis Rio Colorado (SLRC), Sonora, Mexico and Yuma County (YC), Arizona. Epidemiological teams from Mexico and the United States conducted a binational outbreak investigation to confirm this cluster and determine the etiology. We performed additional case-finding and classified GBS cases through interviews and medical record review. To investigate exposures, we reviewed disease surveillance data, performed *C. jejuni* stool culture, conducted a case-control study examining food and water exposures of cases with GBS or *C. jejuni* infection, and performed an environmental assessment of water systems. From May 4-July 21, 2011, 16 SLRC residents and 8 YC residents developed GBS, far exceeding the expected number of cases. Twenty-one GBS patients (81%) reported antecedent diarrhea. Approximately two weeks before this cluster, weekly YC *C. jejuni* reports doubled compared with the 3 previous years. Though *C. jejuni* diagnostics were limited, 2 GBS patients had stool cultures yielding *C. jejuni* and 4 others had positive serologic or stool antigen tests. In the case-control study, all 7 GBS case-patients from YC traveled to SLRC during the exposure period versus 37% of 19 matched controls (mOR: 10.2; CI: 1.4-inf.). Few case-patients or controls (<20%) drank tap water, but >95% reported exposure through other routes. Case-patients consumed more washable, uncooked produce items than controls (Median: 7 vs. 5; P = 0.04). The SLRC municipal water system had a history of inadequate chlorination and pipe disruptions. Inadequately disinfected tap water contaminated with *C. jejuni* was the likely source of this first mainland North American outbreak of GBS. Improved water treatment practices were implemented and the institution of new epidemiological surveillance strategies in SLRC since this investigation will improve early detection of diarrheal outbreaks and facilitate public health actions.

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IDENTIFICATION AND CONFIRMATION THROUGH MULTIPLEX PCR OF THE SPECIES OF *ARCOBACTER* IN ISOLATES FROM HUMAN AND ANIMAL FECAL SAMPLES IN LIMA, PERU

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A multiplex PCR was used to confirm the identity of isolates that are phenotypically suggestive of *Arcobacter* from human and animal stool samples in Lima, Peru. We evaluated 57 bacterial isolates from human fecal samples (3), pigs (52), lion (1) and rabbit (1), with the following phenotypic characteristics suggestive of *Arcobacter*: gram-negative rods, curve-shaped C or S, mobile, aerobic and microaerophilic, circular colonies 1 to 2mm in diameter at 18-24 hours of incubation in microaerophilic conditions on blood agar, non-hemolytic and lactose negative colonies on MacConkey agar, oxidase and catalase positive. The study of genotype was performed by multiplex PCR, as reported previously, using primers targeting the 16S and 23S rRNA genes for the detection of three species*: A. butzleri, A. cryaerophilus, A. skirrowi*, with a molecular weight of 401-bp, 257-bp, 641-bp, respectively. It was confirmed molecularly that Arcobacter was in 87.7% (50/57) of the isolates studied, 90% (45/50) of which corresponded to *A. butzler*, 8.0% (4/50) to *A. cryaerophilus* and 2% (2/50) *to A. skirrowi*. A proportion of 12.3% were negative with the primers used. Of the three human samples, two isolates corresponded to *A. butzleri* and one to *A. cryaerophilus*. The rabbit and lion isolates were *A. butzler*i. Of the pig isolates, 91.1% (41/45) *A. butzler*, 6.7% (3/45) were *A. cryaerophilus*, and 2.2% (1/45) *A. skirrowi*. The presence of *A. butzler*, *A. cryaerophilus* and *A. skirrowi* were confirmed in 87.7% of the total isolates of human, pig, rabbit and lion fecal samples. The remaining 12.3% of the isolates are likely composed of other species of *Arcobacter*.

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RISK PRACTICES REGARDING ANIMAL AND HUMAN ANTHRAX IN BANGLADESH: AN EXPLORATORY STUDY

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From August 2009 to October 2010, there were 14 outbreaks of anthrax in Bangladesh that included 140 animals and 273 human cases. A collaborative team of icddr,b and IEDCR undertook a qualitative investigation to explore livestock rearing practices, the handling of sick and dead animals and the anthrax vaccination programme among outbreak-affected communities as potential contributors to the animal anthrax outbreaks. We conducted a qualitative study in 5 anthrax outbreak-affected villages in 2009 and 2010. To explore butchering sick animals and carcass disposal practices, we conducted in-depth interviews, observation and group discussions with the owners of sick cattle and people who participated in butchering activity. We used key-informant interviews with local government livestock officers to explore the supply and delivery of anthrax animal vaccine. Farmers in these areas raised cattle, goats and/or sheep in their courtyard, fed them dry and green rice straw, green grass gathered from the pastures, rice husk, wheat bran, and oil cake made locally from mustard or sesame seeds. They also grazed the livestock in pastures. Cattle represent a significant financial investment, so when sick cattle were on the verge of death, cattle owners and their neighbors and friends often rapidly slaughtered the cow near or inside the cowshed. The farmers reported discarding the carcasses and butchering waste in nearby ditches, flood waters or open fields. A few carcasses were buried near the owners' house, but often these were dug up by scavenging dogs and foxes. Skinners removed the hides from discarded carcasses and sold them in the local market. Government livestock officers reported that cattle in these outbreak communities did not receive routine anthrax vaccination due to shortage of vaccine and manpower. In conclusion, the slaughter of anthrax infected animals and disposal of butchering waste and carcasses in environments where ruminants live and graze, combined with limited vaccination, provided a context that permitted repeated anthrax outbreaks. Because of strong financial incentives, slaughtering and discarding moribund animals will likely continue. Surveillance for earlier detection of anthrax outbreaks, and better vaccination coverage for at-risk animal population may reduce animal and subsequent human infection.

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EFFECT OF GALANTAMINE ON TULAREMIA PATHOGENESIS IN A BALB/C MOUSE MODEL

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Galantamine is an inhibitor of acetylcholinesterase able to interact with nicotinic acetylcholine receptors as well. Owing to the significant role of cholinergic anti-inflammatory pathways in neuro-immunomodulation, we aimed our effort to examine the effect of galantamine on tularemia-infected BALB/c mice. Animals were infected with *Francisella tularensis* LVS and treated with galantamine in a total amount 0.1 mg/kg of body weight. We examined total mortality, interleukin 6 (IL-6) and interferon gamma (IFN-β levels using enzyme-linked immunosorbent assays using plasma samples. Beside the cytokines assay, the following biochemical markers: inorganic phosphate, uric acid, lactate dehydrogenase, gamma glutamyltransferase, creatinine phosphokinase and amylase were assayed using an automated device. The two opposing processes were proven in the laboratory animals in course of galantamine after tularemia infection: up regulation of IFN-βand down regulation of IL-6. In compliance with expectations, tularemia infection resulted in damage of kidneys, as hyperphosphataemia and hyperuricaemia were proven in the infected animals. Surprisingly, galantamine resulted in calming down of the nephropathy. Markers of kidney dysfunction were modulated as well. The most surprising parameters was alteration of mortality in course of tularemia. As mentioned above, galantamine can significantly influence the immune response. Owing to the results, we infer implication of the cholinergic anti-inflammatory pathway.

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CHARACTERIZATION OF A MURINE MODEL FOR HUMAN SCRUB TYPHUS

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There are over one million scrub typhus cases annually with one billion people at risk of being infected, illustrating the importance of scrub typhus in global health. *Orientia tsutsugamushi*, the etiologic agent of scrub typhus, is a rickettsia transmitted by the parasitic larval stage of trombiculid mites. *O. tsutsugamushi* Karp strain (OTK) has been used extensively in mouse studies using various inoculation strategies with little success in inducing disease progression similar to that seen in human cases, but inducing inoculation route-specific pathology. Intravenous injection of spotted fever and typhus group rickettsiae result in disseminated endothelial infection that causes similar pathology to the human diseases. The objective of this project was to develop a disease model that demonstrates pathology and target cells similar to those of severe human disease. Development of this model will allow for investigation of the immunological mechanisms that mediate protective immunity in scrub typhus infections. This study reports an intravenous infection model under development in our laboratory. C57BL/6 (B6) mice were determined to be susceptible to intravenous challenge by OTK with overt signs of illness with a dose dependent time of onset. Lethal infection occurred after intravenous inoculation of 1.25×10^6 focus forming units (FFU) of OTK with an LD_{50} of approximately 1.25×10^5 FFU. Signs of illness began on day 7 with death occurring ~3-5 days later. Immunohistochemical staining for OTK antigens demonstrated extensive endothelial infection, most notably in the brain and lungs. The histopathology revealed cerebral perivascular lymphohistiocytic infiltrates, focal hemorrhage, meningoencephalitis, and interstitial pneumonia. Intravenous inoculation of *Orientia tsutsugamushi* Karp strain resulted in a disseminated endothelial infection with pathology in B6 mice resembling that of human scrub typhus. A dose dependent severity was also established, providing an avenue to elucidate determinants of severity in scrub typhus infections.

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INVASIVE METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* INFECTIONS AMONG CHILDREN IN BAMAKO, MALI

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Infections due to methicillin-resistant *Staphylococcus aureus* (MRSA) are increasing worldwide. The clinical spectrum of the disease ranges from nasal colonization to superficial and invasive infections. There is a paucity of data about MRSA disease in the pediatric Malian population Hospitalized children aged 0-15 years and ambulatory patients aged 0-35 months evaluated at Hopital Gabriel Toure, the main pediatric hospital in Bamako, with fever ≥39°C or syndrome compatible with invasive bacterial infection were invited to participate in a study in which blood

and relevant body fluid; e.g. cerebrospinal fluid (CSF)) were cultured to identify *S. aureus*; Hospitalized children were followed until discharge and outpatients were contacted if the culture result was positive. From January 2007 to December 2011, 10750 hospitalized children were enrolled, 5600 ambulatory patients were enrolled. MRSA was isolated from 147 inpatients (1.3%) and 23 outpatients (0.4%). Among inpatients, 70 cases (47.6%) occurred in 0-to- 11 month old infants, 19 (12.9%) in 1-t0-4 years old and 58 (39.4%) in 5-to- 15 year olds. 110 isolates (74.8%) were blood only, 15(10.2%) from under skin fluid, 13 from pleural fluid(8.8%), 4 from muscular fluid (2.7%), 3 from joint fluid(2%) and 2 from cerebral spinal fluid(1.3%). 46(31.2%) inpatients died. Among outpatients, 15 (65.2%) cases occurred in infants and 8(34.7%) in 12-to- 35 month olds. 18 isolates (78.2%) were blood only, 4 from muscular fluid (17.3%) and 1 from under skin fluid (4.3%). Follow-up of ambulatory revealed that 15 children had improved, 4 were had not and 4 could not be located. In conclusion, MRSA is common and frequently fatal among children in Mali.

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SALMONELLA MENINGITIS AMONG CHILDREN HOSPITALIZED IN BAMAKO, MALI

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Bacterial meningitis are medical and therapeutic emergency. Three microorganisms (*Salmonella pneumoniae, Neisseria meningitidis*, Hib) are responsible of case majority. Now a day other micro-organisms are observed like *Salmonella*. That why we are doing this study to observe *Salmonella's* part among meningitis epidemiologic. Hospitalized children aged 0-15 years evaluated at Hopital Gabriel Toure, the main pediatric hospital in Bamako, with fever ≥39°C or syndrome compatible with invasive bacterial infection were invited to participate in a study in which blood and relevant body fluid (e.g. Cerebrospinal fluid(CSF)) were cultured to identify *Salmonella*; Hospitalized children were followed until discharged. From January 2008 to December 2011, 7403 hospitalized children were enrolled; *Salmonella* was isolated from 22 inpatients (5.4%). Among patients, 3 cases (13.6%) occurred in 0-to- 1 month old infants, 12 (54.5%) in 1-t0-23 months old, 5 (22.7%) in 24-to- 59 months old and 2(9,1%) in olds > 59 months. *Salmonella* D was the most strain isolated (54,5%) follow by Salmonella spp (22,7%), *Salmonella* B (18,2%),*Salmonella* E or G (4,5%),6(27.3%) patients died. *Salmonella* was most sensible to the ciprofloxacin (100%) and ceftriaxone (95, 5%). The majority of patients (36, 4%) were hospitalized during over ten days. In conclusion, meningitis with salmonellae is common particularly among children from 1 to 23 months old and frequently deadly among children in Mali.

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A COLLABORATIVE RESEARCH AND ADVOCACY EFFORT FOR TYPHOID FEVER CONTROL IN NEPAL: THE PILOT INTRODUCTION VACCINATION PROGRAM

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Typhoid fever continues to be a public health problem in many developing countries. Nepal is one of the highest typhoid incidence countries and infamously referred as "*Enteric fever capital of the world*". Typhoid Vi polysaccharide vaccine has been in market by multiple producers now after the first trial was published in lancet in 1987 by Acharya et al. Typhoid Vi polysaccharide vaccine is known to be safe and moderately efficacious. Few countries (China, Cuba, Vietnam, and the State of Delhi in India) have introduced typhoid vaccine into a public health program. The Vi-based Vaccines for Asia (VIVA) Initiative initiated a joint collaborative effort with the government and non-government partners to introduce typhoid Vi polysaccharide vaccine as part of their public health program. Prior to the start of the program initial meetings were conducted with the health officials to have their opinion on the use of typhoid vaccine in Nepal. Later through qualitative research perceptions of target population and major stakeholders were assessed to guide the formation of a communication strategy to increase awareness about the disease, control measures and importance of vaccination. Regular meetings with health officials, national and international non-for profit organizations, and district education office were conducted in parallel to project field activities. District Lalitpur was chosen for the pilot program of the program. Typhoid fever control has made it's place in the list of government's list of high priority diseases. It has been recommended by the national committee on immunization practices that typhoid vaccines should be used to control the disease. Typhoid vaccination has also be included in the five year health plan of the government. The formal and informal meetings played an important role in the advocacy efforts of creating awareness for typhoid control in Nepal. The government of Nepal has always been committed to control of disease through vaccination, however, the efforts and results from studies conducted under VIVA initiative provided evidence for policy decisions.

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EPIDEMIOLOGICAL AND MICROBIOLOGICAL DIAGNOSIS OF BOVINE TUBERCULOSIS IN SLAUGHTERHOUSES, BURKINA FASO

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Bovine tuberculosis is still unknown in Burkina Faso despite the importance of livestock. The disease is widely suspected at slaughterhouses, but not supplemented by laboratory tests in the country. This study aimed to investigate tuberculosis in bovine carcasses through lesions in lymph nodes or organs at routine inspection, and to identify the causal strains. A prospective study conducted in two slaughterhouses located at Ouagadougou and Bobo-Dioulasso from May-October 2011. A structured questionnaire administered to the owners of suspected carcasses at routine inspection to collect epidemiological data. Sample of lymph node or organ injured collected from suspected animals, and submitted to microscopic examination after Ziehl Neelsen coloration and to bacterial culture using Lowenstein-Jensen ordinary and enriched mediums. From a total of 1499 carcasses examined, 101 showed suspicious lesions of tuberculosis representing a prevalence of 6.07%. The distribution of injuries shows frequently a violation of respiratory lymph nodes with a rate of 92.08%. The isolation and identification of isolates have confirmed 48 positive cultures at a rate of 51.54%. Among these, 47.92% are represented by *Mycobacterium bovis*, 6.25% by *M. africanum*, 4.17% by *M. tuberculosis* and 41.66% by non tuberculosis mycobacteria strain. In conclusion, bovine tuberculosis circulates in Burkina Faso and *M. bovis* is one of potential causative strains of this disease. Other strains of *Mycobacterium* such as M. *africanum* and *M. tuberculosis* were also identified.

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DEVELOPMENT AND VALIDATION OF A QUANTITATIVE PCR (TAQMAN) ASSAY FOR DETECTION AND EARLY DIAGNOSIS OF *LEPTOSPIROSIS INTERROGANS* USING THE JOINT BIOLOGICAL AGENT IDENTIFICATION AND DIAGNOSTIC SYSTEM (JBAIDS)

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Leptospirosis is a potentially serious disease often mistaken for other acute febrile illnesses because of its nonspecific presentation. Current gold standard methods for leptospirosis diagnosis, the microscopic agglutination test (MAT) and an ELISA assay, have several limitations for early diagnosis because of technical complexity and low sensitivity. Diagnostic tests that generate results quickly, cheaply, and definitively are needed. We are addressing this clinical diagnostic need by developing a real-time PCR TaqMan assay using a freeze-dried real time PCR on the Joint Biological Agent Identification and Diagnostic System (JBAIDS) platform for the rapid detection of *Leptospira* bacteria in field samples. Our assay uses primers and probes targeted to highly conserved leptospiral outer membrane proteins (OMPs) on the lipl32 gene. We assessed a 132 bp target for detection of *Leptospira* human pathogenic species using dual-fluorogenic hydrolysis probes. Quantification, accuracy and precision of the assay were determined through serial dilutions of *L. interrogans* serovar Autumnalis genomic DNA, representing a strain of reemerging infectious disease and a major causative agent in Northeast Thailand. We determined assay sensitivity using tenfold serial dilutions in duplicate, ranging from 10 ng to 1 fg. The assay consistently successfully detected as low as 10 fg or 2 genomic (ge) equivalents. The limit of detection was very low at 100 fg or 20 ge. We tested a diverse panel of pathogenic and non-pathogenic *Leptospira* reference strains, genetic near neighbors, and human DNA. Our results suggest this method is a highly sensitive and specific diagnostic tool for identifying pathogenic leptospirosis. Standard diagnostic tests using the MAT and ELISA methods were used to classify serum samples collected in 1999-2002 from patients admitted to a hospital in Kanchanaburi province, Thailand, with a febrile illness and clinical suspicion of leptospirosis. Using the results of these assays, 17.5% of patients were positive for leptospirosis. We are currently evaluating the clinical utility of our JBAIDS assay, compared to the sensitivity and specificity of the standard MAT and ELISA methods, for detecting pathogenic species of Leptospira in these and other clinical samples from Thailand.

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CYSTIC ECHINOCOCCOSIS IN SUDAN AND SOUTH SUDAN: PAST AND FUTURE OF AN IMPORTANT NEGLECTED DISEASE

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Cystic echinococcosis (CE) is a zoonotic disease affecting mainly various species of livestock and humans. It is caused by metacestodes of dog tapeworms of the *Echinococcus granulosus* complex. The metacestodes usually form fluid filled cysts ('hydatids') located in liver, lungs and other organs. CE is distributed world wide, acquiring public health or economic significance in areas where extensive livestock production provides suitable conditions for the cyclic transmission between dogs and other animals which can serve as intermediate hosts. It is considered an emerging disease in many parts of the world, in some regions re-emerging after initially successful control. The global burden of CE is estimated at >1,000,000 DALYs (disability adjusted life years) lost, which gives CE a greater impact than onchocercosis, Dengue fever and Chagas disease, and approaches the burden caused by African trypanosomosis and schistosomosis. CE has been reported from the majority of countries in subsaharan Africa. However, as it is typically a disease affecting pastoral communities which often live in remote areas. reliable data on prevalence of CE in humans or animals are only known from few regions. In livestock, CE seems to be widespread and frequent especially in eastern and southern Africa. In contrast, high-prevalence regions of human CE are focally distributed in some African countries including Southern Sudan. In Sudan, all epidemiological conditions for autochthonous transmission of cystic echinococcosis (CE) are given: In rural areas there are large numbers of dogs in and around villages, and infection can occur with offal from slaughterhouses or during unsupervised home slaughtering. the disease seems to occur sporadically in human in a large part of the country. Nevertheless, even if the parasite may have lower infectivity to humans, the infection can occasionally get established and progress to clinical CE. This study aims at highlighting of the course of research on CE in different animals and humans in Sudan since the disease was first reported in 1908. Recent data about the genetic identification of the parasite in the country in both humans and animals and its importance for future control programs is discussed.

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A CASE OF GIANT SPLENIC HYDATIDOSIS

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Hydatidosis is a parasitic disease caused, in most cases, by a parasite called *Echinococcus granulosus*, this is an endemic disease present in all the continents of the world. In Peru, a South American country, the prevalence of this disease commonly present in the central andes reaches about 9%. Frequently people acquire the disease by the breeding of dogs and the habit of feeding these animals with guts of cattles and sheeps that are infected with the parasite, the Echinococcus can live in the intestines and feces of a contaminated dog, who contaminates the water and food, the eggs from this parasite are accidentally ingested by humans, as a result people acquires the disease. The hydatid cyst in humans is located in the liver at a rate of 70% and in the lung about 30% of the time, the reported prevalence of hydatid cyst of the spleen ranges between 0.9% to 8% of the cases, and as a primary presentation less than 2%, making it quite rare. We present here a case of primary hydatid cyst located in the spleen of a 75 years old Peruvian woman, the diagnosis was made by ultrasonography and computed tomography scan which showed a giant hydatid cystic mass located under the spleen in the left hypochondrium measuring 165 x 130 mm of diameter, the other abdominal and thoracic organs were normal, the western blot test was positive for hydatid disease. The patient was operated laparoscopically without any complications, postoperative evolution was favorable, pharmacological treatment was performed with albendazole 400 mg every 12 hours, in 3 cycles with one-week break between them.

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PHYSIOLOGICAL AND PATHOLOGICAL LIVER PROFILE CHANGES AFTER *IN VITRO* AND *IN VIVO* EVALUATION OF THE THERAPEUTIC EFFECTS OF NEW HETERO-CYCLIC ORGANIC COMPOUNDS AS THIAZOLE, TRIAZOLE, PYRIDAZINE AND PYRIMIDINE, IN COMBINATION WITH ALBENDAZOLE, AGAINST *ECHINOCOCCUS* METACESTODES

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The metacestode (larval) stage of the tapeworm *Echinococcus multilocularis* causes alveolar echinococcosis (AE), a mainly hepatic disease characterized by continuous asexual proliferation of metacestodes by exogenous budding resulting in tumor-like infiltrative growth of the parasite lesion leading to physiological and pathological deterioration of the compressed hepatic cells function. Current chemotherapeutical treatment of AE relies on the use of benzimidazole (albendazole, mebendazole), but these drugs act as parasitostatic rather than parasiticidal, and in case of side effects such as liver toxicity, patients are left without valuable alternatives. New hetero-cyclic compounds as thiazole, triazole, pyridazine and pyrimidine with a documented anti-inflammatory, anti-bacterial and anti-fungal effects have been investigated. All compounds have been evaluated *in vitro* for their anthelmintic activities against *E. multilocularis* metacestodes. Some compounds showed a great nematicidal activity (LC 100) ranging between 0.0005 and 0.002 ug/ml. First the compounds *in vitro* treatment downscaled the transcription of the 14-3-3-pro-tumorogenic zeta-isoform in *E. multilocularis*. Second, scanning and transmission electron microscopy showed that the germinal layer of *E. multilocularis* was dramatically damaged following treatment confirmed by using alkaline phophatase as a marker for metacestode damage. This therapeutic effect of the new synthetic hetero-cyclic organic compounds was dose dependent. Similar results were obtained with *E. granulosus* metacestode. Bioassays were performed in *E. multilocularis* experimentally infected mice treated with the new hetero-cyclic compounds alone, albendazole alone and combination of both. Best results were achieved with a comination of the new synthetic hetero-cyclic compounds and albedazole. This study has shown that hetero-cylic organic compounds as thiazole, triazole, pyridazine and pyrimidine derivatives are promising candidates for the development of new anthelmintic agents with a rapid regeneration and hepatic physiological and pathological functional reformation of a damaged compressed liver tissue caused by the tumor-like infiltrative growth of the parasite hepatic lesions.

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CATHETERIZATION OF GIANT ECHINOCOCCAL CYSTS OF THE LIVER: SINGLE CENTER EXPERIENCE

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Giant echinococcal cysts have a diameter 10 cm, and are usually treated with a surgical approach even when they are CE1 or CE3a because standard PAIR procedures cannot achieve complete solidification . Following the pioneering work by Men *et al*., who treated giant CE1 and CE3a cysts by a modified catheterization technique and left the catheter inside the cavity until daily drainage was less than 10 ml, we used percutaneous catheterization in 6 patients with giant echinococcal cysts of the liver. A 6 or 8 F pig-tail catheter was inserted into the cyst under sonographic guidance in the presence of an anesthesiologist. The trans-hepatic approach was chosen in five cases and the trans-costal access in one case. The Men's protocol was simplified by avoiding the injection of any scolecidal and sclerosing agents as all patients were already receiving albendazole. The mean hospital stay was 6 days (range 3-18). All patients were discharged with the catheter still inserted but disconnected from the collecting bag, and returned to our clinic on average every five days (range 2-15) to drain any fluid that may have re-accumulated. The catheter was left inside the cyst until the output decreased to less than 20 ml and the cystic wall appeared permanently collapsed. For all patients the mean catheterization time was 30 days (range 10-60). All patients except for two in which the catheter slid out accidentally during the hospital stay had a solid (CE4) cyst after a mean follow-up of 8 months (range 0-26) . No bacterial infections were observed. Although these are preliminary results, continuous catheterization seems to be a promising alternative to surgery for the treatment of giant echinococcal cysts of the liver. Studies on larger series are needed.

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CYSTIC ECHINOCOCOSIS CLUSTERS AT HOUSEHOLD LEVEL

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Human cystic echinoccocosis is endemic in many regions including some industrialized countries. Its epidemiology at the community level has been widely studied using either imagenological or serological tools. A known and obvious risk factors is contact with dogs, which in turn get infected because of risk practices (home slaughtering, releasing infected offal into the environment). Whether people living in the same household with a CE case have a higher risk of also having the disease has been suggested but not yet demonstrated. This study aimed to determine if household members or people who live close to a case have a higher probability to have CE. Cluster analysis at household level was preformed with data from a rural endemic community in the Peruvian highlands. The community (330 households, 1380 inhabitants) was divided in 6 sectors, and all inhabitants were invited to participate in the survey. The number of members per house varied from 1 to 8 members. One hundred and ten households participated in the survey, with a coverage of 35% of members per house. Fifteen households have at least one infected member (15/110, 14%) and 3 of them had more than one member. Three sectors had more than one household with infected members. The presence of a CE case is associated to an increased likelihood of having another household member infected

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LOOKING FOR THE HABITUAL INTERMEDIARY HOST OF *ECHINOCOCCUS GRANULOSUS* G6 STRAIN IN PERU

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Cystic echinococosis is a zoonotic disease widely distributed around the world, with sustained endemicity in farming countries like Peru. In the last years, with the development of molecular tools, the identification of *Echinococcus granulosus* strains has acquired importance. Some studies refer that strain could influence disease characteristics (e.g. clinical presentation, organ infected, response to treatment, etc). In Peru, a previous study found that human cases were infected by G1 and G6 strains. Since the reservoir of the G6 strain remains undetermined in Peru, the aim of the present study was to determine if goats are the habitual intermediary G6 host. We collected cysts from goats slaughtered in formal and informal abattoirs located in two coastal cities receiving animals from surrounding endemic areas. Information about involved organ, size of cysts, macroscopical evaluation of cysts stage and involution, as well as goat age, sex and geographical origin were recorded. Isolates were identified by DNA sequencing of the mitochondrial cytochrome c oxidase subunit 1 gene. A total of 71 cysts were collected. Initial analysis in 22 cysts found G6 in 68.18% (15/22) and G1 in 31.82% (7/22) of them. Most cysts (20/22, 90.91%) were located in the lungs and only 2 cysts were located in the liver. The above preliminary findings suggest that goats may as reservoirs for G6 in Peruvian endemic areas. Control strategies may be adapted to these hosts to improve the control of parasite transmission

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A 15-YEAR EXPERIENCE WITH HYDATID DISEASE IN A NON-ENDEMIC REGION

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Hydatid disease is a common zoonosis caused by the larval cysts of *Echinococcus granulosus*. The disease is endemic in parts of the world where there is intimate contact between man and the definitive and intermediate hosts, usually sheep and dogs, but there are no large series from non-endemic regions of the world. We report 37 cases over a 15 year period from a Tropical Medicine Clinic in the Bronx. Of these, 23(62%) were male with a mean age of 44±13 years and mean time from immigration 31±14 yrs. All were immigrants, with the majority from Albania, Yugoslavia, Italy, Macedonia,Yemen, Peru and Tibet. Cysts were located in: liver 31(84%), lung 4(11%), bone 3(8%), brain1 (3%). Of the cysts located in the liver the staging was CE1 2(7%),CE213 (42%), CE3b 4(14%), CE4 7(23%), CE5 (3%), 2 unknown and they were followed for a median of 1.4 yrs (6mo.-16yrs). Two large CE2 cysts treated medically elsewhere presented later with devastating consequences. Complications were seen in 10 (32%) of liver cysts with 3 cystobiliary fistulas, 1 abscess, 5 IVC involvement; one ruptured into the intrahepatic IVC and developed pulmonary embolism and hypertension. Two patients ruptured into the peritoneum spontaneously and one ruptured into the liver after trauma. Three liver cysts penetrated the diaphragm. Of those that underwent surgery, 30% recurred. The 3 patients with bone disease presented with neurologic symptoms or pathologic fracture and despite surgery and medical therapy have not been cured and have chronic, debilitating disease at five years. Two pulmonary cases were treated medically; one ruptured into the mediastinum and another invaded into the ribs. Management of hydatid disease is complex and should be based on staging. Improper management can have devastating consequences; increased education in non-endemic regions is needed.

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HIGH DENSITY FERMENTATION OF EG95 IN *PICHIA PASTORIS*

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This study was conducted to explore the expression and high density fermentation of genetically engineered EG95 gene originating from *Echinococcus granulosus*, which causes cystic echinococcosis (CE) in humans and animals with serious public problems and economic loses. The EG95 gene was codon optimized and truncated for expression in *Pichia pastoris*. The genetically engineered EG95 gene was cloned into *P. pastoris* expression vector pPIC9K to construct recombinant plasmid pPIC9K-EG95 (r pPIC9K-EG95). The rpPIC9K-EG95 was then transformed into *P. pastoris* GS115 cells by electroporation, and stable multicopies of recombinant *P. pastoris* strains were selected by G418 resistance with a concentration of 2 mg/L. SDS-PAGE assay of culture broth from one selected expression strain induced by methanol indicated that the recombinant target EG95 protein was about 14.3 kDa and partially glycosylated with the determination of Endo-H. After purification, the recombinant EG95 protein was finally confirmed by mass spectrography. The culture conditions of recombinant *P. pastoris* for its high density fermentation in fermenter were optimized in 4 test batches, and the optimum pH, cultivation temperature and induction time were 5.5, 28℃ and 96 h, respectively. Methanol was added in different adding rate within different fermentation stage. Samples of culture broth were centrifuged (3000×g, 4℃),and the purity and quantity of the recombinant EG95 protein in the supernatant was determined to be 95.9% and 4.68 g/L. In conclusion, the recombinant EG95 protein could be highly expressed in the selected *P. pastoris* strain in fermenters with the stability and high purity. Our performances in this study will contribute to the development of vaccine against CE.

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GEOGRAPHIC CORRELATION BETWEEN TAPEWORM CARRIERS AND HEAVILY INFECTED CYSTICERCOTIC PIGS

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Neurocysticercosis is a leading cause of preventable epilepsy in the developing world. Sustainable community based interventions are urgently needed to control transmission of the causative parasite, *Taenia solium*, to prevent additional neurologic disease. We examined the geospatial relationship between live pigs with visible cysticercotic cysts on their tongues and humans with adult intestinal tapeworm infection (taeniasis)

in a rural village in northern Peru. The objective was to determine whether tongue positive pigs could indicate high risk geographic foci for taeniasis to guide targeted screening efforts. This approach could offer significant benefit compared to mass intervention. We recorded geographic coordinates of all village houses, collected stool samples from all consenting villagers, and collected blood and examined tongues of all village pigs. Stool samples were processed by ELISA for presence of *Taenia* sp. coproantigens indicative of active taeniasis; serum was processed by enzyme linked immunoelectrotransfer blot (EITB LLGP) for antibodies against *T. solium* cysticercosis. Of 548 pigs, 256 (46.7%) were positive for antibodies against cysticercosis on EITB LLGP. Of 402 fecal samples, 6 (1.5%) were positive for the presence of *Taenia* sp. coproantigens. The proportion of coproantigen positive individuals differed significantly between residents of households with a tongue positive pig (2/36, 5.6%), residents living within 100 meters of a tongue positive pig (2/44, 4.5%) and residents living >100 meters from a tongue positive pig (2/322, 0.6%) (p=0.02). The prevalence of taeniasis was >7 times higher among residents living within 100 meters of a tongue positive pig compared to residents living outside this range (adjusted PR 7.5, 95% CI 1.1 52.2). This finding suggests that tongue positive pigs in endemic communities can indicate geospatial foci in which the risk for taeniasis is increased. Targeted screening or presumptive treatment for taeniasis within these high risk foci may be an effective and practical control intervention for rural endemic areas.

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PHARMACOKINETICS AND TISSUE RESIDUE PROFILES OF OXFENDAZOLE IN PIGS

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Oxfendazole (OFZ) is a benzimidazole antiparasitic agent which is effective against pig cysticercosis when given as a single 30 mg/kg dose, and thus a potential key tool in its control/elimination. Only scarce information about its pharmacokinetics and tissue residues in monogastric animals is available. This study assessed OFZ and metabolites [(fenbendazole sulphone (FBZSO2), fenbendazole (FBZ)] plasma pharmacokinetic and tissue residue profiles after a single oral administration to pigs. Two groups of 24 pigs each were assigned to receive a single oral dose of 30 mg/k of either commercial OFZ formulated at 9.06%, or a locally formulated suspension at 22.5%. Blood and tissue samples were collected over 30 days post-treatment and analyzed by HPLC. OFZ was the main compound recovered in plasma, followed by FBZSO2 and low FBZ concentrations. The area under the curve [AUC0-LOQ] of the commercial formulation was 209.9±33.9 µg·h/ml, with Cmax of 5.40±0.65 µg/ml. The parameters for the commercial formulation tended to be higher than those for the locally formulated suspension. FBZSO2 residue levels were the highest found in muscle (0.68±0.39 µg/g) and fat (0.69 ±0.39 µg/g). In liver and kidney the highest residues corresponded to FBZ (5.29±4.36 µg/g) and OFZ (2.86±0.75 µg/g), respectively. In conclusion, high OFZ concentrations over a long period of time complemented with the recovery of the anthelmintically active compound FBZ in the bloodstream are relevant pharmacokinetic data obtained after OFZ administrations at 30 mg/kg, which correlates with the adequate clinical efficacy obtained in pigs infected with cysticercosis. According to the tissue residue profiles, a withdrawal time of 17 days must be allowed for human consumption at this dose

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MOLECULAR DIFFERENCES AMONG ISOLATES OF *TAENIA SOLIUM* CYSTICERCI FROM DIFFERENT REGIONS OF PERU

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Taenia solium cysticercosis is a neglected parasitic disease that is found worldwide. Previously considered to be tool ready for eradication, efforts have not yet proven to be successful. Molecular methods may provide additional insights to better understand the distribution and dynamics *of transmission of T. solium* cysticercosis. We evaluated three loci of *T. solium* as potential genotyping tools. The DNA of the mitochondrial loci for cytochrome B (CyB) and cytochrome C oxidase subunit I (COI), and a nuclear locus encoding the diagnostic antigen Tsol-14 (Ts14) were amplified and sequence analyzed from a blinded set of cysts from 49 different pigs from four distinct geographical regions of Peru. The nucleotide sequences from Ts14 were conserved among all samples, while sequences of CyB and COI showed sequence polymorphisms and unique SNPs. Concatenated pseudogenes were assembled using the sequences of CyB and COI and used to determine phylogenetic relationships among the samples. Neighbor joining tree analyses showed that the sequences clustered into three major clusters A, B and C, while A had 4 sub-clusters (A1-4). After unblinding of the codes we identified that all samples in cluster B were from pigs from the northern coast, and those in cluster C were animals from the southern highlands. Within cluster A, almost all pigs in the sub-clusters A1and A4 were from the central coast, while one pig from A1 was from the central highlands. All samples in sub-cluster A3 belonged to the southern highland (same region as pigs in C), while A2 had one pig from the northern coast and one from the central highlands. These findings suggest that the CyB and COI mitrochondrial loci could be used as preliminary markers for genotyping and better understanding of the transmission dynamics of cysticercosis. Further work would be needed including analyses of nuclear loci as well as samples from other geographical settings.

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FOCAL LOSS OF VASCULAR INTEGRITY AND EOSINOPHIL INFILTRATION ADJACENT TO *TAENIA SOLIUM* CYSTS ARE EXACERBATED BY ANTHELMINTIC TREATMENT IN PORCINE NEUROCYSTICERCOSIS

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The metacestode stage of *Taenia solium* causes neurocysticercosis (NCC) and muscle infections in humans and pigs, with similar cyst morphology. Using the pig as a model of infection in humans, we investigated the effects of treatment with anthelmintics on vascular integrity and host immune reactions to the parasite in the brain and muscles. We used leakage of Evans Blue (EB), infused intravenously, into the tissue around cysts as an indicator of a breakdown of vascular integrity. We examined histopathological changes after anthelmintic treatment following EB infusions in naturally infected pigs. EB leakage was localized to a small region of some cysts appearing macroscopically as a blue dot and indicating a local breakdown of vascular endothelium. On immunofluorescence microscopy, EB penetration into pericystic tissues was identifiable by red fluorescence. Treatment with praziquantel (100 mg/kg po, once) resulted in an increase (8X) in the frequency of muscle cysts with blue dots. Histopathologically, the blue dots correlated with regions of intense cellular infiltrate with abundant eosinophils (Eos) and mononuclear

cells in the cyst wall and surrounding brain or muscle tissue. Interestingly, a significant proportion of tissue Eos had granular blue staining in unstained sections, suggesting uptake of EB dye (presumably bound to albumin). Immunohistochemical staining for an Eos granular protein (EPX) and simultaneous immunofluorescence imaging confirmed that Eos had, indeed, taken up EB in regions of vascular dye leakage. Examination of cyst structure revealed Eos in the tegument, subtegument and the internal regions of the parasites adjacent to the macroscopically identified "blue dots". Taken together, these data suggest a loss of vascular integrity and interaction between the host immune system and parasite occur focally, and that Eos may play a significant role in the pathology that results from this interaction. These findings have important implications for the pathogenesis of histological damage in NCC and may guide strategies for management of the disease.

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FATTY ACIDS METABOLISM AND UPTAKE IN HELMINTHS, FOCUS ON *TAENIA SOLIUM* TSFABP

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Cysticercosis, caused by *Taenia solium*, is a public health problem in developing countries. During characterization of ESTs in the *T. solium* genome project, the most highly expressed mRNA in the adult worm was found to correspond to a fatty acid binding protein (FABP). The FABPs have been involved in the uptake and transport of long chain fatty acids. A recombinant T.solium FABP (TsFABP) was expressed, and its binding affinities determined by spectrofluorimetry; it showed capability to bind different fatty acids with preference for those saturated. The recombinant TsFABP was found to be associated with host-interacting structures in both cysticerci and adult worm in immunochemistry assays, with βTsFABP antibodies being capable of identifying putative homologues in many Taeniid species. Also, while determining the protective potential of the protein, we found specific βTsFABP antibodies in sera from mice infected with *Taenia crassiceps*, suggesting that the protein homologue is exposed to the host in the course of infection. All this results suggest that TsFABP plays a role in the parasite's establishment in host tissues probably involved in the uptake of host's lipids, or its transportation along the syncytial tissues of *T. solium*, before being metabolized. This, supported by the results from the *T. solium* genome project that showed a limited biosynthetic capability in the parasite, lacking enzymes related to fatty acids and lipids modification, v.gr. elongases and desaturases. It is not clear yet how the parasite obtains the necessary fatty acids and lipids for development and maintenance, being apparently unable to synthesize them. The parasite may take up the necessary enzymes from the host, it has been demonstrated already that the cysticerci incorporates important quantities of host´s proteins; or maybe it takes up the lipids directly from the host. Here we explore the potential mechanisms for this probable lipid uptake, using the data obtained from the *T. solium* genome project, and focusing in the probable role of TsFABP.

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ANTIBODIES TO *TOXOCARA CANIS* IN INDIVIDUALS WITH A SINGLE BRAIN ENHANCING LESION

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Neurocysticercosis and cerebral malaria are the most frequent parasitic infections affecting the central nervous system (CNS). Other parasitic infections can also affect the CNS and are likely underdiagnosed because of poor diagnostic suspicion and the lack of accurate diagnostic tests. As an example, neurotoxocariosis is not often considered in the differential diagnosis in patients with brain inflammatory lesions. *Toxocara* is highly prevalent worldwide, can easily reach the CNS, produces granulomatous lesions, and has been associated with neurological symptoms such as seizures. In this study we evaluated archive samples from a series of 101 patients with a single brain enhancing lesion suspected of neurocysticercosis using a western blot assay (LDBIO Diagnostics, Lyon, France) for antibodies to *Toxocara*. From the 101 patients, 56 were seropositive and 45 were seronegative for cysticercosis on western blot. Seroprevalence for toxocariasis was 75.3% (76/101), 56 of 76 individuals reacted to all four diagnostic antibody bands. There was a trend for patients seropositive for cysticercosis to have lower seroprevalence of anti-Toxocara antibodies (39/56 versus 37/45, OR: 0.496, 95% CI: 0.191-1.287, p = 0.145), and cysticercosis seropositive individuals were significantly less likely to react to all 4 antibody bands (25/56 versus 31/45, OR: 0.364, 95% CI: 0.16-0.829, p = 0.015). This unexpected negative association suggests that a proportion of cases of single brain enhancing lesion, seronegative for cysticercosis, may be due to *Toxocara* infections. Alternatively although less likely, patients may have opposite risk factors causing differential exposure, or cross-protective immune responses.

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HIGH PREVALENCE OF SILENT NEUROCYSTICERCOSIS IN AN ENDEMIC RURAL COMMUNITY IN PERU

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Neurocysticercosis (NCC) is a common helminthic infection of the central nervous system and a leading cause of adult-onset epilepsy in low and middle-income countries. However, few population-based studies have examined associations between neurologic symptoms, serology and radiographic findings, particularly as computerized tomography has typically been limited only to symptomatic people. We conducted a population-based neurologic evaluation in a rural endemic village in northern Peru (Rica Playa, Tumbes, pop. 454) to determine the lifetime

prevalence of epilepsy and severe headache in this community. Our 2-stage evaluation began with door-to-door neurologic screening of all residents ≥2 years old using a validated questionnaire followed by clinical evaluation by a study physician for positives. We also collected a blood sample to detect antibodies against *Taenia solium* cysticercosis using an enzyme linked immunoelectrotransfer blot (EITB LLGP). We then invited all residents ≥18 years old, and any person who was seropositive, to have non-contrast computerized tomography (CT) of the head. Of the 385 residents who provided serum, 142 (36.6%) were seropositive on EITB LLGP. Of the 273 residents who accepted CT scan, 53 (19.4%) had radiographic findings consistent with NCC. All 53 had cerebral calcifications (median no. of calcifications 1, IQR 1-2, range 1-15), 1 had a viable cyst, and 1 had colloidal granuloma. Individuals with brain calcifications were twice as likely to be seropositive than those without calcifications (OR 1.9, 95% CI 1.0-3.6). Of the 403 who participated in the neurologic evaluation, we detected 4 residents (1.0%, 95% CI 0.02-2.0%) with epilepsy and 5 (1.2%, 95% CI 0.6-2.3%) with severe headache. Two of the epilepsy cases were active and 2 were inactive. While the prevalence of neurological cases was too small to examine associations, 3 out of 5 (60%) individuals with headache were seropositive and one (20%) had radiographic findings consistent with NCC; none of the examined individuals with epilepsy was seropositive (0/4), nor had NCC compatible findings on CT (0/3, one person with epilepsy refused CT scan). Exposure to *T. solium* is very common in this endemic community where 1 out of 5 residents had brain calcifications; viable forms were rare. However, the vast majority of people with calcifications were asymptomatic.

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WHAT DOES THE ELECTROIMMUNOTRANFER BLOT TELL US ABOUT CYSTICERCOSIS IN PIGS?

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Taenia solium infection burden at village and individual levels remain elusive. This study aimed to relate Electro Immuno Transfer Blot (EITB) seropositivity and pig infection burden. A total of 476 pigs were sampled from a Peruvian endemic area. Seroprevalence was 60.5 ± 4.5% with statistically higher proportion of positive older pigs (>8 months) than young pigs. The logistic model showed that pigs >8 month of age were 2.5 times more likely to be EITB-positive than ≤8 months. A subset of 84 seropositive pigs was necropsied. Forty-one out of 84 positive pigs were negative to necropsy (48.8%) and 43 (51%) had one or more cysts. The Generalized Estimating Equations (GEEs) were applied to fit an ordinal logistic regression model on the necropsied pigs. The model accounted for household clustering since we had to purchase more than one pig in some houses. The probability of having moderate or heavy infection burden increases proportionally with the number of EITB bands. We also demonstrated that there is a high probability of being necropsy-negative for pigs having less or equal than three EITB bands. Therefore, the EITB might be a measure of exposure rather than a test to determine the real prevalence of cysticercosis infection.

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TAENIA SOLIUM : DEVELOP OF A RAT MODEL OF NEUROCYSTICERCOSIS

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Neurocysticercosis is the most common parasitic disease of the central nervous system (CNS) caused by the metacestode form of the tapeworm *Taenia solium* worldwide. The development of a rat model of Neurocysticercosis (NCC) with *Taenia solium* oncosphere was undertaken to: i) obtain a suitable experimental model for neurocysticercosis, ii) describe the inflammatory process, infection, and iii) describe the host-parasite relationship in rat. The rat Holtzman were intracranially inoculated with oncospheres of *T. solium* at different concentrations (10, 20, 30 and 40 parasites) to induce NCC. They were euthanized at 90 days after the inoculation. Their encephala were removed for the histopathologic analysis. Observations of 90 dpi, included the presence of cysticerci in the brain of the rat in the macroscopic observation, specifically in the rats that were inoculated 20 and 30 parasites. Observations which were corroborated by Westrern blot. and histology. In conclusion, this model resembled the natural infection of NCC using the *T. solium* oncosphere as natural stage of infection and the same species of parasite that causes the NCC. It will aid (i) in understanding disease progression, from the early to late stage of infection (ii) improving and generating new diagnostic tools, and (iii) refining or rationalizing the current treatment of the disease in humans.

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GENETIC DIFFERENTIATION OF *TAENIA SOLIUM* STRAINS FROM DIFFERENT PERUVIAN COMMUNITIES USING MICROSATELLITES

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Cysticercosis, endemic to several developing countries, is a neglected helminthic disease that disproportionately affects people, causing significant chronic neurological disease. In contrast to other taeniid cestodes, knowledge about genetic variation in *Taenia solium* is lacking. This is an important consideration in the epidemiology and transmission of these parasites, since genetic variants may differ in their infectivity, pathogenicity and response to treatment. Until now the studies on genetic variability have been based on molecular markers, such as RAPD, and showed poor diversity. Therefore, it is required a tool of sufficiently high resolution to differentiate strains. The availability of the *T. solium* genome let us demonstrate that microsatellites sequences are broadly distributed in coding and no coding regions. Using bioinformatics tools we identified several microsatellites sequences. Microsatellites were identified from a single locus using Blast and an ad-hoc script developed by Gur-Arie et al. Repetitive motifs of 3-10bp with a minimum of three repetitions were filtered. The markers that showed differences in the motifs between the genomic sequence and the *T. solium* ESTs published in the GenBank. Thirteen markers were selected and further evaluated. These markers

were tested with a sample of 12 tapeworms collected from two distant regions in Peru. Six tapeworms were from Tumbes and six from Puno. Each candidate marker was individually amplified from parasite DNA by polymerase chain reaction. The products were analyzed using the QIAxcel System. The size of the amplified product was used for identification. All fragments were amplified and showed the expected theoretical size. Three of them were polymorphic, SSR1, SSR6 and SSR7. These markers were able to differentiate tapeworms from Tumbes and Puno, which were clustered in two groups. Microsatellites polymorphism showed that *T. solium* has genetic diversity not previously reported, supporting the hypothesis of a genetic recombination process in *T. solium*.

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SPATIAL DISPERSAL OF DENGUE IN TWO URBAN AREAS OF SOUTHEAST ASIA: TESTING MULTIPLE MECHANISTIC MODELS

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Dengue has been endemic in Southeast Asia for decades. In much of the region individuals will regularly suffer twice from the disease by the end of their teenage years. Prevention measures, including the use of insecticides and the targeted destruction of oviposition sites has been hampered by poor understanding of the local dispersion of the virus, especially the relative contributions of human and mosquito movements. To address this gap we collaborated with local hospitals and ministries of public health in Bangkok, Thailand and Cebu in the Philippines to develop models to understand the local spread of the virus. We used the geocoded spatial location of 18,425 patient homes who became sick with dengue between 1994 and 2010. We found a significant risk of finding a second case within a month and up to one km of an initial case relative to that expected given the underlying spatial and temporal distribution of cases. In addition we found a steady increase in the extent of spatial dependence between cases over the time series, potentially indicating increasing mobility of individuals. We used population density estimates from LandScan to build agent based simulations of various parametric and nonparametric dengue transmission processes in both cities. We demonstrated that a gravity model was most consistent with the observed patterns of spatiotemporal dependence with a mean distances of under 100m between sequential cases in a transmission chain. These findings provide key insight into the potential dispersal mechanisms of the disease and provide a guide for the targeting of interventions upon identification of dengue cases.

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DECONSTRUCTING THE PROTECTIVE IMMUNE RESPONSES ELICITED BY A NOVEL DENGUE TETRAVALENT VACCINE IN AG129 MICE

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A formulation of a chimeric dengue vaccine viruses containing the pre-membrane (prM) and envelope (E) genes of serotypes 1-4 expressed in the context of the attenuated DENV-2 PDK-53 genome (DENVax) was tested for immunogenicity and efficacy in interferon receptor knock-out AG129 mice. The vaccine was highly immunogenic and elicited protective immune responses against both wild-type (wt) DENV-1 (Mochizuki strain) and DENV-2 (New Guinea C strain) challenge viruses. To understand better the contribution of each chimeric vaccine in the induction of immune responses and protection afforded by DENVax monovalent formulations were injected in AG129 mice and were shown to elicit robust neutralizing antibody responses to the homologous virus and only limited cross-reactivity to other serotypes. A single dose of monovalent DENVax-1, -2, or - 3 vaccine provided eighty or greater percent protection against both wild-type (wt) DENV-1 and DENV-2 challenge viruses. A single dose of monovalent DENVax-4 also provided complete protection against wt DENV-1 challenge and significantly increased the survival times after challenge with wt DENV-2. Preliminary studies from passive transfer of immune serum suggest a potential role of humoral immunity in protection. Currently, we are evaluating the role of $CD4^+$ and $CD8^+$ T cells in protection against homologous or heterologous DENV challenge. Overall, these data highlight the immunogenic profile of DENVax, a novel candidate tetravalent dengue vaccine that is currently in phase I and II clinical trials. In addition, a better understanding on how DENVax works would greatly facilitate our efforts in developing more effective vaccination strategies against dengue.

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CHALLENGES TO DENGUE REPORTING AND SURVEILLANCE IN TRINIDAD AND TOBAGO: LABORATORY DIAGNOSTICS IN THE ABSENCE OF SUFFICIENT CLINICAL DATA

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The Trinidad Public Health Laboratory (TPHL) receives sera from suspected dengue cases for confirmatory testing by a commercially available dengue-specific IgM ELISA. Samples are typically subjected to point-of-care screening at the sending institutions but are often received by TPHL with incomplete laboratory request forms e.g. no record of number of days post onset of illness (d.p.o.), symptomology or demographics, all crucial for determining the most appropriate screening procedure and generating beneficial surveillance data. We examined the challenge presented to TPHL by reviewing the data accompanying a batch of sera (n = 500) received during the 2011 dengue outbreak, which were also screened by a commercially available NS1 Antigen Capture ELISA kit. Of these, 94% were accompanied by point-of-care screening results. The proportions with other data were: sex 62.2%, age 31.4%, patient address 7.2%, d.p.o 3% and symptomology 9.8%. TPHL screening found 45% IgM+/NS1+, 18% IgM+/NS1-, 11.8% IgM-/NS1+ and 21.4% IgM-/NS1-. Of 19 with inconclusive IgM results, 10 were NS1+ and 9 NS1-. Thus NS1 testing detected 69 positive sera that would have been reported as negative or inconclusive. 18 of these were tested by reverse transcriptase PCR and the presence of dengue virus RNA was confirmed in 11. Using TPHL IgM and NS1 ELISAs as gold standards, the sensitivity and specificity of point-of-care IgM screening were 92.3% and 30.9% respectively, and for point-of-care NS1 screening, 85.5% and 90.9% respectively. Our data highlight the benefit of combining virus specific direct testing methods with indirect testing methods when there is inadequate clinical data. However, due to the number of cases with insufficient data, cost effectiveness would be a concern. For improved efficiency and accuracy of dengue reporting and surveillance in T&T, procedures addressing deficiencies in data gathering and screening services must be adopted at all institutional levels. Additionally the low specificity of the point-of-care IgM screening deserves further investigation.

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THE EFFECT OF ANTIBODIES ON ENDOTHELIAL CELLS DURING DENGUE VIRUS INFECTION

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Primary infection with one Dengue virus (DENV) serotype is proposed to confer lifelong homotypic immunity, but only short-term heterotypic immunity to the other three serotypes. Secondary heterologous infection can result in Dengue Hemorrhagic Fever or Dengue Shock Syndrome (DHF/DSS). Evidence supports a role for pre-existing antibody (Ab) to Dengue virus in DHF/DSS pathogenesis via antibody dependent enhancement (ADE), in which Abs from the initial infection enhance virus infectivity rather than neutralize leading to increased virus uptake into cells. Increased vascular permeability caused by loss of endothelial cell (EC) barrier integrity is a hallmark of DHF/DSS in humans. However, the extent of EC permissiveness to DENV infection and the degree of cell death resulting from DENV infection is controversial, as is the mechanism of hemorrhage associated with DENV infection. We hypothesize that DENV Abs can enhance EC infection leading to loss of EC barrier function and vascular leak syndrome. Anti-DENV human monoclonal antibodies (HMAb) were generated by molecular cloning and characterized based on DENV neutralizing and/or enhancing properties *in vitro*. Human dermal microvascular ECs (HMEC-1) were infected with DENV pre-incubated in the presence or absence of HMAbs known to enhance infection. RNA extracted from infected HMEC-1 cells and supernatants were used to quantify DENV genome copies within the cells and released by the cells, respectively, by qRT-PCR. Infectious virion production was determined by plaque assay. HMEC-1 cells supported direct DENV infection, in the absence of Ab, and enhanced infection in the presence of HMAb, demonstrated by increased viral genome copies within cells. These studies will determine the precise role of DENV Abs in enhancing EC infection and inducing EC pathology. Characterizing the ability of HMAbs from DENV infected individuals to induce EC pathology *in vitro* will provide an understanding of the role of Abs in the induction of DHF/DSS.

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OPTIMIZATION AND VALIDATION OF THE PLAQUE REDUCTION NEUTRALIZATION TEST FOR THE DETECTION OF DENGUE VIRUS NEUTRALIZING ANTIBODIES USED IN SUPPORT OF DENGUE VACCINE DEVELOPMENT

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A reliable testing method that properly measures the immune responses to natural infection and/or vaccination is imperative for public health surveillance and vaccine evaluation. It is anticipated that the neutralizing antibody response against each dengue virus serotype may correlate to protection, and the plaque reduction neutralization test (PRNT) is recognized as the gold standard to measure dengue virus neutralizing antibodies. A factorial design of experiment (DOE) approach was used for the development and optimization of a dengue $PRNT_{50}$ in consideration of WHO guidelines. To generate a robust test method, critical testing parameters included optimal number of days of cell seeding prior to performing the assay, percentage of overlay medium and days of incubation post-infection to generate a robust assay method were evaluated and defined. The optimized $PRNT_{50}$ method was then validated in accordance with International Conference for Harmonization (ICH) guidelines. Intra-assay and inter-assay precision of the dengue $PRNT_{50}$ demonstrated that the titers for 87.5-100 % (14/16 -16/16) and 95-100% (19/20-20/20) of the samples tested for all 4 serotypes were within 3-fold dilution of the median titer. Suitable accuracy and dilutability were demonstrated across targeted dilution (1:4, 1:16, and 1:64) for all 4 serotypes. Assay specificity for each of the 4 serotypes was shown by selectively measuring dengue serotype-specific neutralizing antibodies in the presence of other Flavivirus antibodies that were spiked into the test serum samples. The LLOQ of the assay was challenged and assessed by testing the low titer samples repeatedly 11 times for all 4 serotypes. In summary, the validated dengue $PRNT_{50}$ was demonstrated to be suitable to detect and measure the level of dengue virus serotype-specific neutralizing antibodies in human serum samples with acceptable intra-and inter-assay precision, accuracy/dilutability, specificity, and with a LLOQ titer of 10. This assay is being used to support the worldwide clinical development of the CYD dengue vaccine.

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DEFINITION OF PROTECTIVE VERSUS PATHOGENIC IMMUNE PROFILES AFTER DENGUE VIRUS INFECTION

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As the four serotypes of dengue virus (DENV-1-4) increasingly co-circulate, the risk of severe manifestations of dengue disease is of greater concern. Although half of infections are estimated to be subclinical, nearly one-third of the world's population is at risk of DENV exposure. Through a unique cohort study in Thailand, we were able to obtain blood samples from school-age children before and after the dengue season. A four-fold increase in anti-DENV antibody titer was used to identify individuals who experienced DENV infection during the study. Individuals were further stratified into those who experienced overt dengue illness and those who seroconverted without presenting symptoms. This latter outcome, protection from clinical disease, is one goal of vaccination. Since dengue pathogenesis is thought to be linked to an aberrant immune response, we sought to define the balance between protective and pathological immunity using an *in vitro* stimulated PBMC culture system and a multiplexed analysis of cytokine and chemokine content in supernatants. Pre-exposure PBMC were isolated from fifty-one individuals who subsequently had either subclinical or symptomatic infection. After five days in culture with DENV isolates representing the four serotypes, culture supernatants were harvested and subjected to a multiplexed, bead-based array to quantify the presence of 30 different cytokines and chemokines. The authors will show serotype-specific cytokine levels for individuals according to clinical outcome. Studies comparing individuals with varying clinical outcomes are critical for defining protective versus pathogenic immune profiles. Previous studies have shown that neutralizing antibody titers are a poor correlate of protection from dengue disease in this highly exposed population. These analyses of *in vitro* cytokine responses contribute to the understanding of immunity in natural DENV infection and should help inform future vaccination strategies.

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THE ECONOMIC AND DISEASE BURDEN OF DENGUE IN SOUTHEAST ASIA

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Dengue poses a substantial economic and disease burden in Southeast Asia (SEA). Quantifying this burden is critical to inform policy makers, set policy priorities, and implement disease-control strategies. The few published estimates of dengue burden in SEA are based on one or a few countries and their generalizability is limited by methodological differences. We addressed this need by estimating the economic and disease burden of dengue in 12 countries in SEA, containing 560 million

(m) people. We obtained reported cases from multiple sources, including surveillance data, WHO, and published studies. Underreporting was adjusted using expansion factors (EFs)--multiples of reported numbers--obtained from previous empirical studies in SEA. We conducted a systematic literature review to obtain the direct and indirect costs per dengue episode, by country. We extrapolated unit costs using linear regressions with GDP per capita and type of treatment as independent variables, to complete data for countries with published studies. We estimated the cost per fatal episode based on productivity loss using GDP per capita and life expectancy, and used WHO methodology to estimate disease burden (DALYs). As sensitivity analyses, we used 1,000 Monte Carlo simulations varying EFs, share of hospitalized cases, unit costs, and DALYs per case. We obtained an annual average (years 2000-2010) of 3m dengue cases (1m hospitalized and 2m ambulatory), 6,994 deaths, and a weighted overall EF of 8.4. The annual economic burden (with 95% certainty level) was US$896m ($401m-1,920m) or US$1.60 ($0.72-3.43) per capita. The annual number of DALYs lost from dengue was 292,000 (129,000-612,000), or 521 (230-1,092) DALYs per m inhabitants. Cost per capita was 66% of that previously found for the Americas, but DALYs per m were 6.4 times as high. The burden of dengue would be higher had we considered other economic costs, such as prevention and vector control, and long-term sequelae of dengue. These results suggest that efficient technologies that reduce burden of dengue would be cost effective.

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SEROPREVALENCE OF DENGUE IN UNITED STATES SPECIAL OPERATIONS COMMAND PERSONNEL DEPLOYED TO DENGUE-ENDEMIC AREAS

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Both the endemicity and clinical sequelae of dengue infection are increasing worldwide. The increases are at least in part a result of widespread travel and the increased range of *Aedes albopictus*, a competent vector of dengue. US Army Special Operations Command (USASOC) personnel are at an increased risk of exposure to dengue based on their widespread presence in dengue endemic areas worldwide. Furthermore, repeated deployments to these areas, oftentimes with the same personnel going to different dengue endemic areas sequentially, increase the risk for developing the more serious sequelae of dengue, dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Information about the seroprevalence rate of dengue in USASOC personnel is critical to assessing risk to this population for future deployments and tailoring preventive medical countermeasures and leveraging field diagnostics. In the first part of a two part project to assess baseline seroprevalence of dengue in USASOC units, a random, unit-stratified sample of 500 anonymous serum specimens from personnel assigned to units in USASOC deployed to Latin America from 2006-2008 were screened for dengue antibodies using a microneutralization assay. Of the 500 specimens screened, 56 out of 500 (11.2%) had neutralizing titers (MN50 ≥ 10) against at least one DENV serotype; subsequent positive sample titration resulted in 48 out of the 56 positive samples (85.7%) with NT titers (MN50 ≥ 10) against at least one dengue serotype for an overall dengue exposure rate of 9.6% (48 of 500). Similar results (15/111, 13.5%) were found on subsequent predeployment testing of USASOC personnel in 2012. These findings show that exposures to dengue in USASOC operations are more common than previously thought thus lending increased importance to preventive countermeasures. The significance of these findings with regards to force deployment and personal risk is discussed.

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A MODEL OF TRANSDISCIPLINARY STUDY DESIGN FOR UNDERSTANDING DENGUE TRANSMISSION IN THE DEVELOPING WORLD: APPLICATION OF AN ECOHEALTH APPROACH IN BANGLADESH

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A marked global re-emergence of epidemic dengue reflects the failure of interventions based on traditional reductionistic disciplinary approach to the understanding of dengue disease transmission. By challenging these reductionist notions, the present study asserts the notion that comprehending dengue disease transmission requires application of a holistic, transdisciplinary epistemology that can assess the driving eco-bio-social determinants and their interaction with human action. Developing an appropriate transdisciplinary research design in the context of developing countries requires bringing the knowledge and practice partners together (vertical integration), along with integration of multiple disciplinary areas (horizontal integration), and considerations of social, economic and cultural context without compromising scientific goals. We formulated a multi-tier transdisciplinary study design to apply a holistic "Ecohealth Approach" to understand dengue virus transmission and the dynamics of human choices and preferences concerning this. The study was implemented in Dhaka, Bangladesh, where the 16 million occupants have been exposed to a resurgence of dengue since 2000. To develop a suitable research design, we considered variation in: socio-economic status among the city-zones, gender inequality, population density, housing, and water supply, waste disposal and sewage systems. Multiple disciplinary aspects were encapsulated by examination of: i) rates of human exposure to dengue virus (DENV) by identifying individuals (via a serosurvey in 1200 households) with IgM and IgG antibodies to DENV and acute cases of illness from hospitals (200 diagnostic study of suspected hospitalized patients) by identifying the presence of DENV RNA by PCR amplification procedures; ii) abundance of dengue vector (*Aedes aegypti* and Ae. albopictus) during monsoon and dry seasons in the same households; iii) self-risk perception by the community members, and other patterns of human behavior; iv) human population density, available housing and water and waste supply and disposal systems; and iv) human organizations responsible for interventions. We envision that such a transdisciplinary, holistic epistemological framework will help to determine the eco-bio-social factors responsible for dengue virus transmission, and which can then be apply to the general context of the developing world.

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DEVELOPMENT OF A TARGET PRODUCT PROFILE FOR DENGUE DRUGS

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Dengue fever has serious public health implications for Florida and currently threatens nearly 3 billion people worldwide. The USF Global Health Infectious Disease Research Program (GHIDR), USF Center for Drug Discovery and Innovation (CDDI), Florida's Schools of Pharmacy at UF and USF, and the UF Emerging Pathogens Institute (EPI) have developed a consortium for novel solutions for the detection, prevention and treatment of vector borne diseases. Critical to the consortium's success has been the ongoing development of an initial Target Product Profile (TPP) for potential dengue anti-viral drug candidates. The ideal drug candidate should be active against all known dengue strains, used in primary and secondary infectious independent of serotype, reduces progression to hemorrhagic disease, have a low cost of goods, be fasting acting, rapidly reduce viral burden, and have a safe clinical profile. With no current treatments and only supportive care available, there is no gold standard. Creation of a

unique road-map to druggability represents a daunting challenge. TPPs give researchers a better understanding of existing and future unmet needs for control or possible eradication of dengue. By 'beginning with the end in mind' TPP planning allows for all working areas (e.g. preclinical, clinical, business marketing, distribution and access) to discuss the necessary features critical for end use and reach an initial consensus on the approaches to the milestones and objectives of drug discovery and development. Applicable in all areas of pharmaceutical development, TPPs have the potential to be of even greater impact in tropical neglected diseases.

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VALIDATION OF SURVEY FOR MOSQUITO AVOIDANCE PRACTICES IN INTERNATIONAL TRAVELERS FOR DENGUE PREVENTION

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Dengue is the most common Arboviral infection in travelers to international destinations in tropical and sub-tropical environments. Currently, there is no vaccine or treatment for Dengue; rather, travelers are offered a set of mosquito avoidance practices (MAP) as recommendations for decreasing the risk of infection. In addition, travelers who are 'visiting friends and relatives' (VFR) have a risk of acquiring Dengue equivalent to those living in endemic regions, and an increased risk of severe Dengue if infected during travel. A pilot study was conducted to identify the factors associated with compliance to the MAP guidelines in travelers planning to VFR. A mixed methods survey was developed to correspond with the Precaution Adoption Process Model (PAPM) and tested in travelers to the cultural celebration, Carnival, in Trinidad & Tobago 2012. The survey successfully measured Dengue knowledge, attitudes, intent, motivation and cultural influence on planned travel behavior for MAP. Construct validity and reliability of the instrument was determined by performing an exploratory factor analysis. The resultant Cronbach alpha was .94, indicating internal reliability. The constructs which loaded in the factor analysis were in agreement with the theoretical models developed to investigate the factors associated with compliance to MAP and the PAPM-Dengue model. Further research is necessary to determine if the theoretical models can be used to predict travel behavior; however, the survey can be used as a tool to determine the stage of a traveler within the PAPM-Dengue model. The stages of the model indicates the traveler's level of awareness, perception of Dengue issues, motivation for Dengue prevention at the individual level, and attempts to compliance with MAP recommendations during travel to endemic regions. This can aid in better understanding the social epidemiological aspects of Dengue transmission in travelers.

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DENGUE AND PREGNANCY IN KAMPHAENG PHET PROVINCIAL HOSPITAL

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Dengue, the vector- borne disease is a major problem particularly in tropical region like KPP, Thailand. About 60% of dengue is reported in adults during past few years and the pregnant women are susceptible to dengue infection with high mortality rate. Dengue and pregnancy is rarely reviewed and summarize in Thailand. KAVRU provides the dengue PCR testing in admitted patient at KPPPH for 20 years that help in diagnosis and early treatment. This retrospective review aims to describe the clinical and laboratory data in confirmed cases of dengue and pregnancy. 16 pregnant were diagnosed of dengue from ICD-10 during 2009-2011 but only 13 cases had confirmed dengue infection either by PCR testing or IgM and IgG ELISA level. The result showed the average age of the patient is 22.7 years (range 16-33), mostly are housewife and employee (38% for each group) and in the 3rd trimester of pregnancy (62%), admitted duration after illness onset range from 1 to 6 days. The clinical presentation are Fever (100%), Muscle/Body pain and Malaise/Fatigue (85%), Headache, loss of appetite and skin rash (70%), Nausea(62%), Vomiting and abdominal pain (54%), others are cough (46%), Joint pain (38%), sore throat, diarrhea and eye pain (23%), Three of the patients were bleeding (2 -epistaxis and 1 melena) . None has neurological involvement. The 2 hDF, 3 DHF I, 5 DHF II and 3 DSS were classified. The PCR on admission day revealed 3 DENV-1, 6DENV-2, 1DENV-4 and 3 negative. The serologies were 10 acute secondary dengue infection. Three of the patients were in labour; 2 were normal labour and 1 was cesarean section due to twin pregnancy. All infant had good Apgar score without fever. From this data, we can summarize that the warning signs in non-pregnant and pregnant are the same but obviously the most symptoms in pregnant are fever with muscle pain and malaise more frequently than gastrointestinal symptoms and this can imply to guideline for dengue with pregnancy and lead to vertical transmission detection.

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DENGUE EPIDEMIOLOGY AND ASSOCIATED FACTORS IN RICE FARMERS OF ENDEMIC REGION OF PERUVIAN AMAZONIAN

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Dengue fever is an endemic infection in the Peruvian Amazonian and has become a public health problem. We conducted a seroepidemiological cross-sectional study in rice farmers of the Alto Mayo Valley. The objective was to determine the prevalence of IgG and IgM antibodies against dengue and to identify the factors associated with positive serology. 250 farmers were enrolled, the prevalence of IgM antibodies (recent infection) was 7.25% (95%CI: 3.79-10.6). IgG antibodies (past infection) prevalence was 62.4% (95%CI: 56.2 - 68.6). None of the participants with recent infection presented suggestive symptoms of dengue. Only two of the 18 participants with recent infection had headaches, sore calves, back pain, abdominal pain and 3 had pain joints. However, the presence of these symptoms was not associated with dengue infection. In bivariate analysis, farmers older than 30 years (OR= 2.52; 95%CI: 1.22-5.22; p=0.01), residence time more than 25 years in the area (OR=2.19; 95%CI: 1.29-3.72; p=0.004) and farmer working more than 15 years (OR=1.83; 95%CI: 1.06-3.18; p=0.003) have associated with past infection. Age, gender, years of residence in San Martin region, floor material of house, store water inside the home, bird breeding, home proximity to drains, time of work as farmer and elevated floor from the ground were included in the multivariate analysis. Only the residence time of more than 25 years in the region was associated (adjust OR=1.88; 95%IC: 1.04-3.38). In conclusion; dengue virus is circulating in the region affecting more than half of enrolled farmers. The infection is mainly asymptomatic in acute cases. Time of residence in this region for more than 25 years was associated with past infection of dengue, which suggests the existence of multiple factors or cultural, home and works characteristics and that they have not been explored in our research.

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EVALUATION OF THE PERFORMANCE OF CLINICAL AND LABORATORIAL DENGUE DIAGNOSIS DURING AN EPIDEMIC IN A MEDIUM-SIZED CITY IN SOUTHEAST BRAZIL

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Acute dengue disease may present with symptoms that overlap with other febrile diseases, making it impossible to reach the diagnosis based solely on clinical grounds.The WHO definitions indicate that a probable dengue case is characterized by fever and two of the most common dengue symptoms, such as rash, aches and pains, and a positive tourniquet test, and a confirmed case as the one fulfilling the above mentioned clinical criteria associated to virus isolation, a positive PCR, and either IgM or IgG seroconversion in paired samples. However, since viral isolation and performing PCRs need specialized laboratories, most of the time the dengue diagnosis is performed by detecting either, or both, NS1 antigen and dengue-specific IgM antibodies. In Brazil, when dengue incidence rates are over 300 cases/100,000 habitants, dengue cases are no longer confirmed by laboratory tests and every clinical suspected cases are confirmed as dengue cases if they fulfill the WHO criteria for the probable dengue case. In order to assess the performance of the clinical-based diagnosis in epidemic situations, a convenience sample of clinically diagnosed dengue cases were blindly tested by NS1 antigen and dengue-specific IgM antibody detection, according to the manufacturers' protocols. These acute phase samples (up to 5 days of disease onset) were collected on the last week of April and first week of May of 2011 in Ribeirão Preto, a city located in Southeast Brazil and that experimented a huge dengue-1 outbreak, with 21,142 confirmed dengue cases, most of them based on the clinical manifestations. A laboratory confirmed dengue case, used as gold standard for the diagnosis, was defined when a sample was positive for either NS1 antigen or IgM-antibody detection alone or when both were positives. Out of 1,490 suspected dengue cases, dengue diagnosis was determined in 1,148 and in 969 by clinical/epidemiological and laboratory evaluation, respectively. The sensitivity, specificity, positive and negative predictive values were 82.6%, 33.4%, 69.8% and 50,9%, respectively. Thus, it is clear from this work that a clinically-based diagnosis is highly sensitive but it is not very specific, resulting in an overestimation of the real dengue cases. Finally, this study shows that a more reliable dengue-specific test is urgently needed, even though recently, NS1 antigen detection has been used to confirm dengue cases during the acute phase of the disease.

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DENGUE VIRUS INFECTION OF U.S. MILITARY SERVICE MEMBERS FOLLOWING DEPLOYMENT TO DENGUE ENDEMIC REGIONS

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Dengue virus is the most important arthropod-borne viral disease worldwide and a significant infectious disease threat to deploying military personnel. Although it has been found to be a cause of febrile illness and lost duty time since the Spanish-American War, the epidemiology of infections in contemporary worldwide military missions has not been described. To characterize the risk of dengue infection on deployment, pre- and post-deployment banked serum from 1000 Service Members who deployed to dengue endemic regions were tested for the presence of neutralizing antibody against all four dengue virus serotypes to identify Service Members who were infected during deployment. A 7.6% global seroprevalence was found post-deployment; regionally, 12.4% prevalence was found among deployers to South America, 7.2% to Southeast Asia, 6.0% to Africa, and 4.8% to Central America. Odds of experiencing fever during deployment were more than three times greater among those with dengue antibodies post-deployment than those without. Demographic data from post deployment questionnaires, to include age, gender, branch of military service, and military occupation, were analyzed for association with dengue seroprevalence. Age was found to be a risk factor, whereas combat and law enforcement occupations were found to be protective. These results confirm that dengue infection is a military threat in our current theaters of operation. Further, this will help guide medical threat planning and better describe risk factors for infection among adults from non-endemic populations deploying to endemic regions.

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EVALUATION OF THE PERFORMANCE AVAILABLE DENGUE DIAGNOSTIC TESTS: PROGRESS TOWARD AN OPTIMAL DIAGNOSTIC ALGORITHM

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Dengue diagnostic testing has been problematic because no single test detects a single diagnostic analyte with the sensitivity required to confirm the diagnosis using a single specimen obtained during the febrile phase of the illness. However, use of two diagnostic tests; one to detect dengue virus (DENV) RNA or antigen (NS1), and one to detect IgM anti-DENV confirms a high proportion of persons with dengue. This study was conducted to provide an estimate of the performance of dengue diagnostic tests during the first 14 days after onset of fever and to develop a diagnostic testing algorithm for single specimen testing. The longitudinal course of the illness was reconstructed using a panel (n=1234) of archived acute and convalescent serum samples obtained from previously confirmed dengue cases on days 0-14 days after their onset of symptoms (DPO). The panel contained all four DENV serotypes and cases occurred in Puerto Rico between 2005 to 2010. Specimens were tested by CDC developed qRT-PCR and IgM anti-DENV tests and commercial ELISAs for NS1 antigen and IgM anti-DENV. The results from this evaluation provided the most robust DENV laboratory testing algorithm in which end-users have a comprehensive set of results of test sensitivity, specificity and confidence intervals in relation to DPO. In addition, an analysis of the sensitivity of these tests in relation to one another (i.e. qRT-PCR sensitivity in serologically confirmed cases; or IgM sensitivity in qRT-PCR-positive cases by serotype) was determined.These results will be used to develop an algorithm of diagnostic testing to provide the highest likelihood of a definitive result in a single serum/plasma specimen obtained during the acute phase of dengue (DPO= 0-14) in persons with dengue as defined by the 2009 WHO Dengue Case Classification.

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IMPLICATIONS OF POPULATION STRUCTURE FOR GENETIC STUDIES IN DENGUE

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Dengue virus infection is globally the most common mosquito-borne infection after malaria, although only a small percent experience the more serious form of disease. Recent studies in Thai and Taiwanese pediatric populations demonstrated a strong association of dengue hemorrhagic fever (DHF) with the gene CD209, and with JAK1 in a Brazilian largely

adult population from Salvador (n=522). Also, the gene IL28B has been shown to be strongly associated with clinical presentation and treatment outcome of the flavivirus, hepatitis C. Despite laboratory and literature support, none of these genes were associated in a recent GWAS study of dengue shock syndrome (DSS) in Vietnam. In order to test these candidates in a different Brazilian population, 620 subjects from the city of Fortaleza (1000 km north of Salvador) were genotyped at 730 SNPs in the type I IFN response pathway genes and other potential candidates. Both samples were community-based and mirrored the census characteristics for adults in these cities. We failed to see an association for any of the 3 candidates with DHF. Further analysis revealed a surprising degree of city-specific population structure despite the high degree of admixture in Brazil. We performed analysis with the program Structure as well as principal components analysis (PCA) to determine the proportion of contribution from African and European ancestry for all individuals based on pseudoancestors from the HapMap database. Data sets for Native Americans were not available to us. Although both cities are administratively considered The Northeast, we found that they demonstrated 3-fold difference in African genetic contribution (Salvador>Fortaleza) and behaved as distinct populations. Using genomic control (GC) analysis, we found that the population homogeneity was greater for Fortaleza than Salvador, though both had β(inflation factor) values near 1. Values close to 1 indicate little structure. When analyzed together, βwas 1.4. In the Americas where admixture is the rule rather than the exception, studies may fail to find true associations (or find spurious ones) even in the same geographic region or country because of stratification. Unless the gene effect is very large and the mutation very old, association across widely separated populations will be difficult to establish. Either some of these associations are spurious, are of small effect or there are population- and age-specific genetic associations.

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HOW CAN THE TRENDS IN INCREASING SEVERITY OF DENGUE EPIDEMICS BE REVERSED - TAIWAN'S EXPERIENCES TO GLOBAL CONTROL

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Although epidemics of dengue/dengue hemorrhagic fever (DHF) have occurred more than about sixty years since the outbreak of DHF exploded in the Philippines, the severity of dengue epidemics involving many DHF and fatal cases have increased in recent two to three decades in Asia and South American countries. The reasons to this trend in increasing severity of dengue epidemics include global warming, increases in rapid travel, mosquito breeding sites and population movement, failure in effective prevention and control and the selection of dengue virus with greater epidemic potentials. However, what are the mechanisms involved in this increasing epidemic severity through population levels and the selection of more virulent dengue viruses (DENV) over time in the same epidemic in one area or cross-country spread at molecular and cellular levels have remained unclear. In Taiwan, most epidemics of dengue started from imported dengue viruses. However, DHF cases only occurred in certain years. Since dengue has not become endemic/hyper-endemic yet, most epidemics are caused by a single serotype of dengue virus, and each suspected dengue case has to be reported with specimen taken and laboratory confirmation, all these together provide the best chance to understand the epidemiological characteristics between mild clinical form (dengue fever, DF) versus severe clinical form (dengue hemorrhagic form, DHF). We have used geographical information system (GIS) and molecular investigation to find the changes of quasispecies of DENV population in different cases within a family over time. Recently, we examined both dynamic changes of viral and immunological attributes along the epidemics and found that both viral and immunological factors are involved in the selection of DENV through a series of interactions between DENV and host responses.

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THE POTENTIAL ECONOMIC VALUE OF A DENGUE DRUG

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There are currently no approved drugs or vaccines to treat dengue fever. Drugs that prevent the progression of uncomplicated dengue to more severe disease (DHF and DSS) have the potential to significantly reduce morbidity and attendant health care costs. While a tetravalent dengue vaccine is likely to be approved in 2015, and this will be an important advance in the prevention of dengue, it is unlikely to result in the cessation of dengue transmission in the short term. Dengue drugs, if they become available, will offer a complementary approach to the management of the disease. This presentation will, for the first time in the public domain, describe the global economic burden of dengue, the likely impact of vaccine approval on future dengue case loads, the potential approaches to pricing and the future market for a dengue drug.

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IMMUNOGENICITY AND SAFETY OF INACTIVATED CHROMATOGRAPHICALLY PURIFIED VERO CELL-DERIVED JAPANESE ENCEPHALITIS VACCINE IN CHILDREN

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Japanese encephalitis (JE) is a common cause of viral encephalitis in Asia which can be controlled by safe and effective vaccines. Inactivated mouse-brain derived vaccine has a worrisome safety profile while live attenuated vaccine cannot be used in immunodeficient individuals. This study aimed to evaluate the immunogenicity and safety of inactivated chromatographically purified Vero cell-derived JE vaccine (ICVJEV, Beijing P-3 strain) among healthy children. One hundred and fifty-two healthy Thai children aged 1-3 years with no history of JE vaccination received 3 doses of ICVJEV (Liaoning Cheng Da Biotechnology Co., Ltd, China) on Day 0, 7-28, and 365. JE virus neutralizing antibodies (JE $PRNT_{50}$) using Beijing P3 strain was measured at the Center for Vaccine Development, Mahidol University, in sera samples collected on Day 0, 1 month after the 2nd vaccination, 1 year, and 1 month after the 3rd vaccination. Adverse events were observed for 28 days after each vaccination and serious adverse events were monitored throughout the study. There were 152 enrolled subjects, 79 were male and 73 were female. The mean age was 14.4 months (SD 3.8 months). On Day 0, 5 subjects (3%) had detectable neutralizing antibody levels over the seroprotective level (> 1/10 dilution). One month after the 2nd vaccination, all subjects (100%) had anti-JE level higher than the protective level (GMT 150). Seroprotection rate 1 year after the 2nd vaccination and 1 month after the 3rd vaccination were 89.2% (GMT 49.3) and 100% (GMT 621.7), respectively. The local adverse events included tenderness (0.5%), redness (0.5%), ecchymosis (0.2%). Systemic reactions included fever (17.6%), vomiting (8%), poor appetite (5.3%). No vaccine-related serious adverse events were noted. In conclusion, Inactivated chromatographically purified Vero cell-derived JE vaccine is

safe and immunogenic. It resulted in 100% seroprotection and provided high geometric mean titers after the 2rd and 3rd doses of vaccination. The seroprotection rate after 2 doses at 1 year was also high (89.2%).

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AN OVERLOOKED METHOD OF DIAGNOSIS: TROPICAL VIRUSES ISOLATED FROM PHARYNGEAL SWABS

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Tropical viruses, such as dengue virus (DENV), Venezuelan equine encephalitis virus (VEEV), Group C viruses, and hantaviruses cause febrile disease in Peru. Their diagnosis traditionally relies on assays_for example, isolation, PCR, or serology_performed on blood samples. Only rare case reports describe the identification of DENV and VEEV in respiratory samples, and this has never been reported for Group C viruses. As part of an infectious disease surveillance network performed in collaboration with the Ministry of Health of Peru, we collected serum samples from patients presenting with acute febrile disease and tested them using culture or RT-PCR. In addition, in those presenting with respiratory symptoms, an oropharyngeal swab was obtained and initially evaluated for the presence of influenza virus. In order to investigate the sensitivity of pharyngeal swabs in detecting tropical viruses, we retrospectively tested swabs collected from patients with established infection based on their serum sample. Viral isolation and/or RT-PCR was performed on pharyngeal swabs from the following number of patients with established viral infections: DENV, 117; VEEV, 4; and Group C viruses, 1. Virus was detected by viral isolation in 28 (24%), 4 (100%), and 1 (100%) for DENV, VEEV, and Group C viruses, respectively, while PCR detected DENV in 27/77 (35%) and VEE in 4/4 (100%). Also, we identified Rio Mamore virus, a hantavirus previously not associated with human disease, using PCR performed on a pharyngeal swab specimen obtained from a febrile patient. Although potentially not as sensitive as blood specimens, pharyngeal swabs may provide a useful alternative sample collection method to obtain specimens in populations (such as children) that are difficult to obtain blood specimens from.

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GENOMIC AND PHYLOGENETIC CHARACTERIZATION OF BRAZILIAN YELLOW FEVER VIRUS STRAINS

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Globally, yellow fever virus infects nearly 200,000 people leading to 30,000 deaths annually. Although the virus is endemic to Latin America, only a single genome from this region has been sequenced. Here we report 12 Brazilian yellow fever virus complete genomes, their genetic traits, phylogenetic characterization, and phylogeographic dynamics. Variable 3' non-coding region (NCR) patterns and specific mutations throughout the open reading frame altered predicted secondary structures. Our findings suggest that whereas the introduction of yellow fever virus in Brazil led to genotype I a predominant dispersal throughout South and Central Americas, genotype II remained confined to Bolivia, Peru and the western Brazilian Amazon.

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BAYESIAN PHYLOGEOGRAPHIC RECONSTRUCTION USING AFRICAN YELLOW FEVER VIRUS ISOLATES INDICATES RECENT SIMULTANEOUS DISPERSAL OF EAST AND WEST LINEAGES

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Yellow fever virus (YFV) is a mosquito-borne flavivirus that is a major public health problem in tropical areas of Africa and South America. There have been detailed studies on YFV ecology in West Africa and South America but current understanding of YFV circulation on the African continent is incomplete. This inadequacy is especially notable for East and Central Africa, for which the unpredictability of human outbreaks is compounded by limitations in both historical and present surveillance efforts. Sparse availability of nucleotide sequence data makes it difficult to investigate the dispersal of YFV in these regions of the continent. To remedy this, we constructed Bayesian phylogenetic and geographic analyses utilizing 49 partial genomic sequences to infer the structure of YFV divergence across the known range of the virus on the African continent. Relaxed clock analysis demonstrated evidence for simultaneous divergence of YFV into east and west lineages, a finding that differs from previous hypotheses of YFV dispersal from reservoirs located on edges of the endemic range. Using discrete and continuous geographic diffusion models, we provide detailed structure of recent African YFV lineage diversity. Significant transition links between extant East and West African lineages are presented, implying connection between areas of known sylvatic cycling. Multiple demographic models reinforce the existence of a stably maintained population of YFV with spillover events into human populations occurring periodically. The layered modeling approach used in the study demonstrates the recovery of ecologically and historically significant structures in the dataset. Presented results justify further incorporation of Bayesian phylogeography into GIS analyses as an augmentation to the study YFV ecology and human disease risk.

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PROGRESS TOWARDS THE CONSTRUCTION AND APPLICATION OF MODOC/WEST NILE AND MODOC/CULEX CHIMERIC VIRUSES FOR THE IDENTIFICATION OF GENETIC ELEMENTS THAT MODULATE FLAVIVIRUS HOST RANGE

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Most known flaviviruses, including West Nile virus (WNV), are maintained in natural transmission cycles between hematophagous arthropods and vertebrate hosts. In contrast, other flaviviruses such as Modoc virus (MODV) and Culex flavivirus (CxFV) have host ranges restricted to vertebrates and insects, respectively. The genetic elements that condition these differential host ranges and transmission cycles have not been identified. To address this issue, we are developing chimeric viruses between MODV and WNV as well as MODV and CxFV. Briefly, studies are underway to replace the capsid, pre-membrane and envelope (C-E) genes as well as the pre-membrane and envelope (prM-E) genes of a full-length MODV infectious clone with the corresponding regions of WNV and CxFV. Fusion-PCR will be used to construct junctions between the 3'UTR of MODV and capsid genes of WNV and CxFV as well as the junctions between the envelope genes of WNV and CxFV and the adjacent NS1 gene of MODV. The *in vitro* growth properties of the MODV/WNV and MODV/CxFV chimeras will be compared to those of the parental viruses, and these data will be used to design additional chimeras that contain other genetic regions of the heterogeneous viruses. Overall, these chimeric viruses will provide useful tools for the identification of the genetic determinants that modulate the flaviviral host range.

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WEST NILE IN BULGARIA LABORATORY CONFIRMED CASES

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West Nile virus (WNV) is a mosquito-transmitted arbovirus belonging to the genus *Flavivirus* in the family *Flaviviridae*. WNV has a wide geographical range, including Europe, Asia, Africa, and Australia. WNV first appeared in the U.S. in 1999 in New York City. Recently a WNV outbreak occurred in Romania affecting 57 human cases. Samples from acute febrile illness cases (AFI; n=400) were collected from different hospitals in Bulgaria. Clinical and epidemiologic findings, as well as acute and convalescent blood samples were collected from all patients. Samples were tested for WNV among a panel of other potential AFI viruses using IgM and IgG ELISA (Focus Diagnostics, Cypress, CA, USA). Positive WNV IgM cases were further confirmed using indirect fluorescent antibody (IFA; Euroimmune, Germany), PRNT; according to CDC guidelines and rRT-PCR. Two patients had anti-WNV IgM, as determined by ELISA and IFA, and their PRNT was positive at a dilution of 1:20 or more. Both patients had fever and headache; one patient, living in a village bordering Romania, also exhibited neurologic manifestations during the WNV outbreak in 2010. West Nile virus is circulating in Bulgaria. The clinical presentations for these patients were consistent with case reports previously described. A regular surveillance plan of the mosquito population in Bulgaria should be implemented as an early warning system. Further studies are needed to determine the strain of the circulating WNV. This represents the first laboratory confirmed cases of West Nile virus infection in Bulgaria.

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EVOLUTIONARY DYNAMICS OF WEST NILE VIRUS IN THE US: DIFFERENTIAL ANALYSIS OF THE PHYLOGENY AND SELECTION PRESSURE IN HUMANS, BIRD AND MOSQUITO HOSTS

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West Nile virus (WNV) is a mosquito-borne virus that is maintained in a bird-mosquito enzootic cycle, but it can also infect other vertebrates including humans. Phylogenetic analyses identified two major WNV genetic lineages: lineage 1 (composed of clades 1a, 1b and 1c) and lineage 2. Recently 3 additional lineages have been described. WNV was first reported in the US in 1999. In 2001, a new WNV genotype (WN02) appeared in the US, displacing the ancestral genotype NY99 by 2003. The WN02 genotype became dominant in the US due to its ability to disseminate more efficiently in domestic mosquitoes as compared to genotype NY99. To date, all strains circulating in the US belong to Clade 1a of lineage 1. In this study we performed evolutionary analyses in WNV isolates circulating in the US. We examined the genetic variation in the open-reading frame (ORF) of 29 selected WNV strains obtained from blood donors during the US epidemics from 2006-2011 and all sequences available in the GenBank (1999-2009) from bird and mosquito-origin. We used maximum-likelihood and Bayesian approaches for phylogenetic analyses and HyPhy for selection pressure analyses. Besides identifying the two main WNV genotypes present in the US (NY99 and WN02), phylogenetic analysis shows that the latter is sub-divided into three groups (g1, g2 and g3). A new sub-type within g3 emerged around 2003 in Southwestern US (SW/WN03). Within this sub-type, we identified a cluster with strains derived from blood donors and birds from the states of ID and ND detected during 2006-2007 (NW/WN06). Few nucleotide and amino acid changes are responsible for the emergence of new US sub-types. We detected a number of codons subjected to positive pressure in structural and non-structural protein genes. Viral adaptation through fixation of spontaneous mutations is an important factor potentially associated with reoccurrence of WNV outbreaks in the New World. The emergence of new genetic variants of WNV raise issues of public health importance because they may affect the sensitivity of both screening and diagnostic assays, as well as the development of vaccines and drugs.

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PRODUCTION OF NON-PING PONG DEPENDENT PIWI RNA-LIKE SMALL RNAS IN THE MOSQUITO MIDGUT IN RESPONSE TO WEST NILE VIRUS INFECTION

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Small RNA regulatory pathways are an integral component of endogenous transcriptional regulation, as well as innate immunity. The role of small-interfering RNAs (siRNAs, 20-23 nts) in response to viral infection in invertebrates has been extensively studied and well-characterized. However, the extent to which other small RNA populations participate in antiviral defense is comparably less understood. Recent evidence has suggested that components of the PIWI class of nucleic acid binding proteins can also participate in antiviral defense in mosquitoes. The hypothesis for this observation is that the PIWI-interacting RNA (piRNA) pathway may act as a compensatory response to viral infection when the RNAi pathway is deficient or overburdened. Primary piRNAs show significant strand bias, and range from 24-30 nts in length. Recent studies with Chikungunya virus and Sindbis virus (*Togaviridae*) in *Aedes aegypti* and *Ae. albopictus* mosquitoes and cell lines have described the production of viral-derived piRNA-like small RNAs that exhibit "ping-pong" amplification. In this model, primary piRNA transcripts with a strong bias for a 5′ uridine terminus bind target transcripts and result in cleavage of the target 10 nucleotides upstream from the 5′ uridine residue. Due to this, secondary piRNAs produced in this manner exhibit an adenine residue in the 10 position. Here we characterize 24-30 nt piRNA-like small RNAs produced in response to West Nile virus (*Flaviviridae*) infection in *Culex quinquefasciatus* mosquitoes. Interestingly, while exhibiting a strong bias for sense-strand orientation, viral-derived piRNA-like RNAs did not exhibit signatures indicative of ping-pong amplification. Previous studies of dengue virus-infected *Ae. aegypti* support this observation, suggesting that the piRNA pathway may function differently in response to flaviviruses compared to alphaviruses.

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URBAN-RURAL DIFFERENCES IN THE IMMUNE GENE EXPRESSION PROFILE OF GHANAIAN CHILDREN

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Urbanization is having dramatic effects on disease patterns in developing countries. However, little is known about corresponding immune system changes associated with this process. A key mechanism underlying immune response is variation in gene expression which is controlled by genetic as well as environmental factors. Exploring immune gene expression patterns between urban and rural populations could provide insights into the impact of urbanization on changes in the immune profile as well as on disease outcomes. Our aim was to determine whether different geographical environments in one region of Ghana impacted on mRNA expression levels of selected immune genes. Our study population was 151 children aged 5-13 years attending rural, urban low socioeconomic status (SES) and urban high SES schools in the Greater Accra Region of Southern Ghana. Parasitological samples were collected to detect helminth infections and malaria parasites. Gene expression was examined in *ex vivo* whole blood samples using real-time quantitative

PCR. Selected polymorphisms of the IL-10 gene as well as selected polymorphisms of Toll-like Receptor (TLR) genes were also genotyped using the MassARRAY system. *S. haeamatobium* infection was detected among rural but not urban children. Intestinal helminths were found in all three areas but were highest among rural subjects followed by the urban low SES school and lastly the urban high SES school. Malaria was prevalent in the rural area and negligible in the urban area. Marked differences in gene expression were observed between the rural and urban areas as well as within-urban variations based on socioeconomic level. Current *S. haeamatobium* infection modulated the expression of some genes involved in the TLR signalling pathway and accounted for the urban-rural differences found with respect to these genes. Interestingly, IL-10 gene expression was elevated in the rural compared to urban area ($p<0.001$) but this was not associated with current helminth infection. In addition, elevated IL10 mRNA levels were influenced by genetic polymorphisms in the IL10 gene but these did not explain the urban-rural differences observed. In summary, we ascertained that immune gene expression patterns are strongly influenced by environmental determinants and can serve as important markers of the effects of urbanization on immune profile changes within the tropics.

1110

THE *LOA LOA* GENOME AND ITS IMPLICATIONS FOR THE *WOLBACHIA*-FILARIA SYMBIOSIS

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Loa loa, the African eyeworm, is the least well-studied of the human pathogenic filarial parasites but is gaining clinical prominence because of serious adverse events following antifilarial treatment. Furthermore, unlike any of the other filarial parasites of humans, *L. loa* does not contain the alpha-proteobacterial *Wolbachia*, considered to be an obligate endosymbiont of most filarial worms. Therefore we generated a 20x draft genome sequence of *L. loa*, and a 12x draft genome of the related filarial parasite *Wuchereria bancrofti* for comparative purposes. The *L. loa* genome assembly is 91.4 Mb with a scaffold N50 of 172 Kb, making it the most contiguous filarial genome assembly to date. RNA-Seq data generated from *L. loa* microfilariae was used to generate a high-quality annotation of the *L. loa* transcriptome, which is predicted to encode 14,925 genes. Gene order is highly conserved between *L. loa* and the related parasites *W. bancrofti* and *Brugia malayi*. Using orthology to *C. elegans*, we profiled the metabolic biosynthesis and transport capabilities of the three filarial worms, in addition to five other free-living and parasitic nematodes. All nematodes showed remarkable conservation of metabolic pathways, with the exception of purine biosynthesis which has been lost independently in multiple lineages. Despite lacking intracellular *Wolbachia*, *L. loa* shows no new metabolic synthesis or transport capabilities relative to the other filarial parasites, and no evidence of extensive lateral gene transfer from *Wolbachia* or from other bacteria. These results suggest the metabolic role of *Wolbachia* symbionts is likely more subtle in nature than providing otherwise unobtainable nutrients.

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IMPROVED QPCR ASSAYS FOR DETECTING FILARIAL DNA IN BLOOD OR MOSQUITOES

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Molecular detection of filarial DNA can be used for mapping endemic areas and for monitoring elimination programs. We previously reported highly sensitive TaqMan qPCR assays for detecting *Wuchereria bancrofti* (Wb) and *Brugia malayi* (Bm) DNA in blood and mosquitoes. The current study compared the performance of qPCR with two different master mixes, namely TaqMan (Reagent A) and SsoFast (Reagent B). Reagent B contains a unique Sso7d fusion polymerase that increases the efficiency and speed of PCR reactions compared to the Taq polymerase present in the TaqMan mastermix. qPCR assays for Wb, *Dirofilaria immitis (Di), D. repens* (Dr) and Bm DNA employed primers and TaqMan probes specific for LDR, MTR1, MTR2, and HhaI- repeat DNA sequences, respectively. The efficiencies of all of these qPCR assays with Reagent A or B were close to 100%. The analytical sensitivity for detecting DNA was the same with either assay for Wb and Di, but the Reagent B assay was more sensitive for Dr and Bm DNA. The detection limits for Wb LDR plasmid DNA and for Di, Dr and Bm genomic DNA were approximately 0.01fg, 100fg,1fg, and 1fg, respectively. Cycle threshold values with reagent B were 2-10 cycles lower (4 to 1000 fold more sensitive) than with Reagent A. qPCR assays for Dr and Bm DNA with Reagent B were more sensitive than assays for Wb and Di DNA with either Reagent A or B. All qPCR assays with Reagent A or B master mixes were species-specific, with no signals detected with DNA templates from other filarial nematodes, *Plasmodium falciparum*, *Aedes aegypti*, or *Homo sapiens*. We compared the performance of the two Wb qPCR assays with field samples from a Wb endemic area. Results were the same with both assays for 50 pools of gravid mosquitoes collected in Wb-endemic areas (24 positive pools) and for 50 human blood samples (17 positives). 15 of 15 dog blood samples with Dr microfilariae were also positive by qPCR with both mastermixes, and no false positive results were observed with uninfected dog blood samples. Additional studies are needed to evaluate the Reagent B qPCR assays with other field samples (blood and mosquitoes). Reagent B costs much less than Reagent A, and this impacts the total cost per PCR reaction. Thus Reagent B qPCR assays provide significant advantages for probe-based qPCR assays for detecting filarial DNA in blood samples and in mosquitoes.

1112

UNRAVELLING THE MUTUALISTIC SYMBIOSIS OF *WOLBACHIA* AND THE FILARIAL NEMATODE *BRUGIA MALAYI*: A SYSTEMS BIOLOGY APPROACH

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The parasitic nematode *Brugia malayi* is a causative agent of lymphatic filariasis, a disfiguring disease affecting over 120 million people worldwide. *B. malayi* exists in a mutualistic symbiotic relationship with *Wolbachia*. Larval development, embryogenesis and adult worm survival are the key biological processes, entirely dependent on this symbiosis. We have applied an omics approach to investigate the molecular basis of this symbiosis. In order to study the role in larval and embryonic development, Illumina RNA Seq was used to produce a comprehensive transcriptome of four time points spanning the early post-infection development of *B. malayi* in the mammalian host *Meriones unguiculatus* and at multiple time-points post-antibiotic depletion from adult females. *Wolbachia*/worm ratios within developing larvae and adult worms at each time point were also monitored by qPCR and fluorescent microscopy. In parallel proteomic profiling of these selected nematode life cycle stages has been adopted to monitor protein expression of *Wolbachia* and *B. malayi*. In-solution tryptic proteolysis coupled with reversed phase liquid chromatography and analysis by high-resolution mass spectrometry provides a powerful tool for global proteome analysis. This initial shot-gun approach has been optimised to include an extensive pre-fractionation step to delve deeper into the proteome by increasing peptide and protein identification. Following basic analysis through established bioinformatics pipelines, transcriptomic, proteomic, and published datasets will be integrated in a systems biology approach with the objective of understanding the molecular basis of the mutualistic *Wolbachia*/*B. malayi* symbiosis.

1113

DEMONSTRATION MIRNA-MEDIATED REGULATION OF EXPRESSION WITH TRANSIENTLY TRANSFECTED *BRUGIA MALAYI* EMBRYOS

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miRNAs have been implicated in transcriptional, post-transcriptional and translational regulation in gene expression. Recently, deep sequencing experiments have demonstrated the presence of miRNAs in parasitic nematodes, including *Brugia malayi* and *Ascaris suum*. However, little is known concerning the role that these miRNAs play in regulating gene expression in these organisms, or the mechanisms through which they might exert their effects. As a first step in exploring the role of miRNAs in *B. malayi*, the target sequence for the *B. malayi* mir-71 miRNA was inserted into the 3′ untranslated domain of a reporter construct consisting of the *B. malayi* HSP70 promoter driving the expression of a luciferase reporter gene containing the first intron of the native BmHSP70 gene and the BmHSP70 3′ UTR. mir-71 was chosen for this study because deep sequencing revealed that it was a very abundant endogenous miRNA. Insertion of the mir-71 target sequence resulted in a decrease in reporter luciferase activity in embryos transfected with this construct to 20% of the level seen in embryos transfected with the un-mutated construct. Mutation of the 3′ end of the mir-71 target (corresponding to the putative mir-71 seed sequence domain) restored the reporter activity to 80% of that seen in with the wild type construct. Similarly, mutation of the 5′ end of the target site resulted in reporter activity which was 50% of that seen with the wild type. In contrast, mutation of the putative slicer recognition site of the mir-71 target sequence did not result in any restoration of activity. These data suggest that the mir-71 miRNA is capable of interacting with and reducing protein expression from mRNAs containing its target sequence. This study further demonstrates that transient transfection of *B. malayi* with constructs containing reporter genes can be used to explore the function of miRNAs in the human filarial parasites.

1114

NOVEL INHIBITORS OF THE *BRUGIA MALAYI* STRESS-ACTIVATED PROTEIN KINASE, BM-MPK1

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Filariasis, caused by thread-like nematode worms, affects millions of individuals throughout the tropics and is a major cause of acute and chronic morbidity. Filarial nematodes effectively evade host immunological responses and are long lived within their hosts. In particular, filarial parasites are particularly resistant to oxidative stress generated by reactive oxygen species (ROS) generated during infection by cells of the innate immune system. We have previously characterized a stress-activated protein kinase, Bm-MPK1, from the filarial parasite *Brugia malayi*, which is related to the evolutionarily conserved human mitogen-activated p38 protein kinase (p38) and the *Caenorhabditis elegans* PMK-1 protein kinases. We have demonstrated inhibition of Bm-MPK1 activity using a panel of known p38 inhibitors. Furthermore, treatment of *B. malayi* adult and larval forms with p38 inhibitors in the presence of ROS compromises the ability of the parasite to effectively mount an anti-stress response leading to the death of the parasite. Inhibition of this pathway may have therapeutic benefit in treating filariasis by increasing the sensitivity of filarial parasites to ROS and other reactive intermediates. We now report on the results of a Bm-MPK1 kinase screen using a focused kinase library of p38 inhibitors. The initial screening plate was comprised of 40 compounds covering 8 structural series. After two follow-up rounds of screening, covering an additional 74 compounds in 3 series, a lead series was selected. Several inhibitors were identified exhibiting potencies ranging from 14-116 nM and exhibiting a 2-5-fold selectivity for Bm-MPK1 over human p38. Several compounds were effective in blocking parasite responses to ROS. Two compounds in particular, Compound 001 & 002, effectively induced parasite death in the presence of ROS at concentrations of ~ 5uM. These results indicate the potential for development of Bm-MPK1 selective inhibitors as anti-filarial therapeutics.

1115

ANTI-*WOLBACHIA* CONSORTIUM (A·WOL) DRUG DISCOVERY: SCREENING DIVERSITY LIBRARIES TO DISCOVER NOVEL AREAS OF CHEMICAL SPACE WITH ANTI-*WOLBACHIA* PROPERTIES

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There is an urgent need to develop new drugs for onchocerciasis and lymphatic filariasis treatment and control. The Anti-Wolbachia Consortium (A·WOL) is testing a diverse range of compounds to find new chemical space to meet this demand. 558,000 compounds have been procured from the following libraries: Medicines for Malaria Venture (MMV - 500,000 compounds), BioFocus® (10,000 compounds), and Shanghai Institute of Materia Medica (SIMM - 48,000 compounds). 12,400 of these compounds have already been screened with 130 hits identified as reducing *Wolbachia* levels by >90%. Hits are scrutinised to assess suitability for further assessment of structure-activity relationships and select the best candidates to take forward as part of the drug discovery program. We are exploring a range of cheminformatic approaches to allow us to rapidly identify groups of compounds that show anti-*Wolbachia* activity and reject those where the chemical space is largely redundant.

1116

EVALUATION OF IGG4 IMMUNE RESPONSES AGAINST OV-16 AND BM-14 IN NON-HUMAN PRIMATES INFECTED WITH *ONCHOCERCA VOLVULUS*

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We studied the temporal evolution of immune responses from six chimpanzees inoculated with 200-400 L3s from *Onchocerca volvulus*. Serum samples and skin snips were systematically collected on a monthly basis for 4-5 years post inoculation. Patent infections were confirmed by detection of microfilaria (Mf) in skin snips. Serological IgG4 responses were evaluated by ELISA against recombinant antigens OV-16 (onchocerciasis) and Bm-14 ((lymphatic filariasis [LF]). Mf were detected at ≥11 months post-inoculation (mean=485 days, median=406, range 336-763 days). Positive immune responses against Ov16-IgG4 were observed before Mf were detected (mean=453, median 462 days), There were no significant differences between detection of Mf and OV-16 seroconversion (p=0.53, paired t-test) showing that both methods have similar temporal diagnostic value. Anti-Bm14-IgG4 responses were always detected after Mf positivity (mean 670 days, median 601), however these differences were not statistically significant (p=0.18). The cross-reactivity with Bm14 is important in areas where onchocerciasis and LF are co-endemic. Our findings suggest that serological evaluations may need an additional highly specific LF antigen to discriminate between onchocerciasis and LF. Further

population-based studies are required to confirm the reactivity of sera from onchocerciasis patients with Bm-14, and the potential use of this antigen as a pan-filarial screening reagent.

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FILARICIDAL DRUGS INDUCE APOPTOSIS IN THE FILARIAL NEMATODE-*BRUGIA MALAYI* AND THIS EFFECT IS NOT PRIMARILY DUE TO *WOLBACHIA* DEPLETION

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Filarial nematodes harbor *Wolbachia* bacteria and depletion of *Wolbachia* in filarial nematodes results in defects in nematode development, fertility and viability. Induction of apoptosis in *Brugia malayi* upon *Wolbachia* depletion was shown and thus suggested the involvement of factors originating from *Wolbachia*, as reported previously. To further confirm that apoptosis occurring during anti-filarial drug treatment is caused by *Wolbachia*'s depletion, we compared two types of drugs: a macrofilaricidal drug which doesn't target *Wolbachia* such as flubendazole and gentamycin, an antibiotic known to be ineffective in killing *Wolbachia* vs. tetracycline, which targets *Wolbachia*. Adult *B. malayi* worms were cultured *in vitro* and treated with 40 µg/ml of tetracycline, 20 µg/ml of flubendazole or 40 µg/ml of gentamycin. As a control for effects of death, we used untreated adult worms that were killed by freezing. Tetracycline and flubendazole killed *B. malayi* adult worms on day 5 and day 6 of treatment, respectively where as with gentamycin treated worms survived for 10 days and untreated worms survived for more 2 weeks. Treated worms were harvested at the point of death or alive (untreated worms) and were sectioned and stained for *Wolbachia* and a Tunnel assay was performed to detect apoptosis. Interestingly, minor degree of apoptosis was already found in untreated worms. These untreated worms harbored many *Wolbachia* based on immunhistology and qPCR. In untreated worms killed by freezing also showed signs of apoptosis although *Wolbachia* could be detected in these worms. In tetracycline treated worms, there were extensive signs of apoptosis and there was also a marked reduction in staining for *Wolbachia*, however, worms treated with flubendazole although showed extensive apoptosis numerous *Wolbachia* could be still be detected. Apoptosis was also observed in gentamycin treated worms while numerous *Wolbachia* were still detected. Thus our results show that induction of apoptosis following anti-filarial drug treatment is mainly caused by worm death due to the drug's activity and its toxicity and is not necessarily due to the depletion of *Wolbachia* alone, as proposed earliar.

1118

IN 2012, CARTER CENTER ASSISTED RIVER BLINDNESS PROGRAMS HALTED OVER 1.2 MILLION IVERMECTIN TREATMENTS IN FOUR COUNTRIES AFTER TRANSMISSION INTERRUPTION WAS DEMONSTRATED

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The Carter Center (TCC) assists national ministries of health in 11 countries in Africa and the Americas to conduct health education and distribute the medicine ivermectin (Mectizan®, donated by Merck) for onchocerciasis. Where possible, programs aim to break the transmission of onchocerciasis as well as control the associated morbidity. TCC partners include national governments, WHO/PAHO, Gates Foundation, Lions Clubs, CDC, and several universities. TCC assisted elimination efforts have been based on more intensive use of ivermectin, with twice or even four times per year treatments, and (in Uganda), vector control. Complete elimination of Onchocerca volvulus is the goal in six countries in the Americas, Uganda and Sudan. Four programs (Mexico, Guatemala, Sudan and Uganda) interrupted transmission in five transmission zones in 2011, which has resulted in withdrawal of an estimated 1,235,000 treatments in 2012. These programs have followed the 2001 WHO guidelines for documenting elimination of onchocerciasis as applied by in the Americas by the OEPA and Pan American Health Organization, and by the ministries of health of Uganda and Sudan. The include demonstration that OV16 antibody tests are < 0.1% positive in children, and PCR assays in vectors demonstrate fewer than 1/2000 infective black flies. In African programs, TDR guidelines are also included where skin snip surveys must show microfilaria (mf) prevalence are less than 5% in all sampled communities (and less than 1% in 90% of sampled communities). TCC assisted transmission zones where treatment is being withdrawn will now enter three years of post treatment surveillance (PTS).

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ROLE OF INDUCIBLE NITRIC OXIDE SYNTHASE (INOS) IN MICE WITH EOSINOPHILIC MENINGITIS CAUSED BY *ANGIOSTRONGYLUS CANTONENSIS* INFECTION

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Nitric oxide (NO) levels in the CSF was found to be a useful prognostic marker in bacterial and tuberculous meningitis, but few studies were conducted in eosinophilic meningitis. Inducible nitric oxide synthase(iNOS) was found to have a detrimental role in the pathology resulting from acute cerebral injury. Inducible NOS was also found to mediate hippocampal caspase-3 activation in pneumococcus meningitis. However, iNOS knockout mice infected with M. tuberculosis developed serious clinical manifestations and granulomatous lesions containing tubercle bacilli throughout the meninges, all of which were absent in wild-type mice. This indicated that importance of NO in defense against TB meningitis. In this study, we used the inducible nitric oxide synthase (iNOS) knockout mice to analyze the brain pathological and apoptotic changes in mice with eosinophilic meningitis. Wild type and iNOS knockout mice were orally infected with 50 *A. cantonensis* L3 via an orogastric tube after slight ether anesthesia and then 6 mice were sacrificed every week for 4 consecutive weeks after infection until the end of the study. Serial brain protein homogenates were used for analyzing the apoptotic protein changes by western blot analysis. Hematoxylin-Eosin staining of brain and TUNEL assay were done for comparing the pathologic changes in wild type and knockout mice. It was found that the iNOS knockout mice had more severe cerebellar inflammation as evidence by the more severity of eosinophilic meningitis, granulomatous encephalitis and perivascular cuffing in cerebellum. By using TUNEL and western blot analysis, it was found that the iNOS knockout mice had more severe apoptosis changes in cerebellum compared to the wild type mice following different weeks of infection. In conclusion, we found that the iNOS is probably protective against parasitic associated eosinophilic meningitis.

1120

THE IMPACT OF A SCHOOL-BASED HYGIENE, WATER QUALITY AND SANITATION INTERVENTION ON SOIL-TRANSMITTED HELMINTH REINFECTION: A CLUSTER-RANDOMIZED TRIAL

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Periodic chemotherapy is a cost-effective approach to reducing soil-transmitted helminth infection. However, without exposure mitigation, reinfection can occur rapidly. We conducted a cluster-randomized trial to assess the impact of a school-based hygiene promotion and sanitation program on reducing infection with soil-transmitted helminths (STH) following a school deworming campaign. Reinfection with soil-transmitted helminths was quantified twice over two years following a baseline measurement and deworming. Forty government primary schools from three administrative districts in Nyanza Province, Kenya were randomly selected and assigned to intervention or control arms. The intervention included latrine construction, provision of handwashing stations, and hygiene education. A random selection of 2,904 school pupils at three time points were assessed for prevalence and intensity of STH infection using stool samples. Observations were conducted at the pupils' homes to assess household water, sanitation, and hygiene conditions and socio-economic status. The impact of the intervention on the prevalence of *Ascaris lumbricoides* was found to be significant for girls (Odds Ratio [OR] 0.49, 95% confidence interval [CI] 0.25-0.98), but not for boys (OR 0.98, 95% CI 0.52-1.88); the effect on intensity of infection followed a similar pattern. There were no significant effects of the intervention on the prevalence and intensity of *Trichuris trichiura* or on the prevalence of hookworm. For the intensity of hookworm infection, stratification by gender revealed a significant impact among boys (IRR 0.21, CI 0.08-0.57) and a trended, though non-significant increase on girls (IRR 2.12, CI 0.86-5.20). Children with lower levels of water, sanitation, and hygiene access at home benefitted more from the school-based program. Provision of school-based sanitation, water quality, and hygiene improvements may reduce reinfection of soil-transmitted helminths following broad-based deworming. The magnitude of the effects may be gender- and worm-specific, pointing to behavioral characteristics associated with reinfection. That poorer children and those with lower access to improved WASH benefitted more from the program has important implications for the equity of WASH provision and health improvement.

1121

EVIDENCE THAT MULTISECTOR FOOD SECURITY INTERVENTION PROGRAM IN RURAL PANAMA REDUCES HOOKWORM INFECTION IN PRESCHOOL CHILDREN

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Since 2007, a multisector food security program in rural Panamanian communities has attempted to improve food security and health through agriculture extension, training in maternal-child nutrition and hygiene, and community organisation. Although gastrointestinal parasites are recognized as a health problem among young children, they have been outside the scope of the program. Our objective was to compare prevalence and intensity of intestinal parasitic infection among preschool children (6-60 mo) not yet involved in the program and those involved for 1 or 5 years. Fecal samples from 153 children were examined for Protoza (direct smear) and helminths (FLOTAC), and household demographics, hygiene behaviours, and sanitation and water infrastructure were noted. An improved household water source was found in 43% of households and was more common where the program had begun in 2007 ($p = 0.0003$). Latrines were found in 82% of homes and 59% of caregivers reported that their child always wore shoes. Hookworm was found in 31% of children, *Giardia* in 28% and *Ascaris* in 16%. Stepwise multiple regression models revealed that hookworm epg was higher in older children, households with more preschool children, and households without an improved water source ($p = 0.0001$). *Ascaris* epg was higher in children with younger caregivers and in households with more children 12 years and younger ($p = 0.02$). Presence of *Giardia* was not correlated with any of the measured demographic, behaviour or infrastructure variables. The number of years involved with the program did not emerge as a factor contributing to either prevalence or intensity of any of the infections. Our results show that that improved water source is associated with reduced hookworm infection but that long-term participation in this multisectoral program alone is not sufficient to protect against gastrointestinal parasites. Our future work will explore whether specific aspects of this multisector intervention are effective in reducing transmission of gastrointestinal parasite infections.

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OXANTEL PAMOATE: REVIVAL OF AN OLD DRUG

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Recent estimates suggest that 4.5 billion people are at risk of soil-transmitted helminthiasis (STH) with over 1 billion people currently infected with one or more of the three common soil-transmitted helminths (i.e. *Ascaris lumbricoides*, hookworm and *Trichuris trichiura*). The global strategy for the control of STH is preventive chemotherapy, however, only a handful of drugs are available against STH, i.e. albendazole, mebendazole, levamisole and pyrantel pamoate). It is recognized that none of the drugs are efficacious against all three STH species with particularly low cure rates observed against *T. trichiura*. While, individually, compounds may not meet the desired target product profile, combination of drugs with different characteristics may achieve higher levels of efficacy then the individual products, which might prolong the useful lifespan of the existing drugs by conferring mutual protection against resistance. We studied the trichuricidal potential of the "old", veterinary drug oxantel pamoate and the effect of oxantel pamoate combined with albendazole, mebendazole, levamisole, pyrantel pamoate and ivermectin *in vitro* and *in vivo*. We calculated an ED_{50} of 4.7 mg/kg for oxantel pamoate against Trichuris muris in mice. For comparison, albendazole, mebendazole, levamisole and pyrantel pamoate are characterized by ED_{50} values of 345 mg/kg, 79 mg/kg, 46 mg/kg and > 300 mg/kg. Oxantel pamoate combined with levamisole and combinations of oxantel pamoate with pyrantel pamoate behaved antagonistically. Highly synergistic effects (combination index <1) were observed when oxantel pamoate-mebendazole was administered *T. muris* infected mice. For the combination oxantel pamoate-albendazole a nearly additive behavior was determined *in vivo*. In conclusion, our study confirmed the excellent trichuricidal properties of oxantel pamoate. Further preclinical studies are warranted with the two lead candidate combinations oxantel pamoate-mebendazole and oxantel pamoate-albendazole.

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TRENDS IN INTESTINAL PARASITISM IN A MILITARY POPULATION IN THE PERUVIAN AMAZON, 2003-2011

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In the Amazon Basin, intestinal parasitism is endemic and is a major cause of morbidity in military and civilian populations. We investigated the trends in intestinal parasitism in a military population operating in the Peruvian Amazon to help guide diarrheal disease treatment and deworming efforts. From 2003 to 2011, all asymptomatic individuals newly stationed at the

Vargas Guerra Army Base in Iquitos were invited to provide a stool sample upon enrollment in a longitudinal study of diarrheal disease. At least one species of intestinal parasite was identified in 74% (3284/4406) of stool samples, and 50% (1650/3284) of infected individuals tested positive for multiple parasitic species. The most prevalent parasites identified were *Ascaris lumbricoides* (40%), *Entamoeba coli* (35%), *Trichuris trichura* (19%), *Uncinaria stenocephala* (16%), and *Giardia lamblia* (16%). The prevalence of intestinal parasitism increased from 67% in 2003 to 81% in 2011 (Odds Ratio (OR)=1.23, 95% CI= 1.19, 1.28; p<0.001), and the probability of multiparasitism among parasite-infected individuals also increased over time (OR=1.15, 95% CI=1.11, 1.19; p<0.001). These trends persisted even after adjustment for age, living quarters, and provenance. These changes in the prevalence of parasitic infection appear to be driven by significant increases in the prevalence of *A. lumbricoides* (OR=1.03, 95% CI=1.00, 1.06), *T. trichura* (OR=1.14, 95% CI=1.10, 1.18), and *G. lamblia* (OR=1.07, 95% CI=1.03, 1.11), as the prevalence of all other parasites tested either decreased or remained stable over the study period. These data demonstrate the high prevalence of intestinal parasitic infection in the Peruvian Amazon and the need for further investigation of their impact on troop readiness in the region.

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COMPARISON OF THE FECAL PARASITE CONCENTRATOR METHOD TO KATO-KATZ FOR THE DIAGNOSIS OF SOIL TRANSMITTED HELMINTHS

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The World Health Organization (WHO) recommends that the Kato-Katz (KK) method be used to diagnose soil transmitted helminth (STH; *Ascaris sp.*, *Trichuris sp.*, and hookworm) infection. However, stool samples analyzed using KK must be evaluated within 24 hours after the sample is produced, creating difficulties during field studies. In settings where laboratory diagnosis is not readily available, alternative techniques that allow stool preservation for transport and later examination are needed. The objective of this study was to compare the sensitivity of the KK method with a formalin preservation technique known as the Mini Parasep® Fecal Parasite Concentrator (FPC) method. In the absence of an accepted "gold standard" test, we will use latent class analysis to compare the results of each test in identifying *Ascaris sp.*, *Trichuris sp.*, and hookworm infections. In 2010, we collected stool samples from 324 residents ≥ 1 year old in randomly selected households in the Guatemalan county of Nueva Santa Rosa. Part of each sample was preserved in formalin in the field using a pre-filled Mini Parasep® Fecal Parasite Concentrator container and the remainder was left unpreserved. Samples were stored in a cooler and transported to the lab during one of two trips each day, and then tested by both methods. KK testing was performed following the WHO recommended procedure, and FPC using the Mini Parasep® protocol. Seventeen (5.3%) individuals tested positive for *Ascaris sp.* by FPC, while 28 (8.6%) tested positive by KK. Three (0.9%) individuals tested positive for *Trichuris sp.* by FPC, while 16 (4.9%) tested positive by KK. One person (0.3%) tested positive for hookworm, which was found by FPC but not KK. Latent class analysis will be utilized to further compare the results of the FPC and KK methods. Although the KK method poses logistic challenges for field surveys, the alternative formalin-preservation FPC method is less sensitive compared to the KK method for the 3 pathogens tested. New diagnostic tools for STH that combine the ease of the FPC method and the sensitivity of the KK method are needed.

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HEALTH-SEEKING BEHAVIORS AND TREATMENT FOR SOIL TRANSMITTED HELMINTH INFECTION IN NUEVA SANTA ROSA, GUATEMALA - 2010

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Infection with a soil-transmitted helminth (STH) (e.g., *Ascaris sp*. and hookworm) can lead to dysentery, anemia, and cognitive impairment, especially in children. Annual or bi-annual treatment with antihelminthic drugs has been shown to reduce infection intensity in affected individuals. To prevent STH-associated morbidity and mortality, the Guatemalan Ministry of Public Health and Social Welfare conducts a semi-annual school-based mass drug administration (MDA) for STH throughout the country. This study examines STH treatment practices and health-seeking behaviors for STH infection in the Guatemalan county of Nueva Santa Rosa (NSR). We collected stool samples from 324 residents ≥ 1 year of age in randomly selected households in NSR between July -August 2010. Samples were tested using both Kato-Katz and Mini Parasep® Fecal Parasite Concentrator methods. Individuals positive for any of the three STHs on either test were considered infected. STH prevalence was 13.3% (N=43/324). STH burden was highest in preschool-aged (PSAC) (7/54, 13.0%) and school-aged children (SAC) (20/104, 19.2%); 9.6% of adults were infected. Overall, 110 (34.1%) individuals reported taking drugs to treat intestinal worms during the previous year; this proportion did not differ significantly between infected and uninfected individuals (p=0.71) though both PSAC and SAC were significantly more likely to receive antihelminthic drugs compared to adults (OR: 4.4, 95% CI: 2.2-8.9; OR: 2.3, 95% CI: 1.3-4.2; respectively). Of those taking drugs, 43.3% (N=45) obtained them at a store or pharmacy, and 37.5% (N=39) from a hospital or clinic. Observing worms in stool in the previous year and regular school attendance were not associated with STH positivity on univariate analysis. Multivariable logistic regression modeling will be used to examine significant risk factors for STH infection in this sample. Treatment with antihelminthic drugs was infrequent in this sample, even among SAC, where only 40% reported receiving treatment in the previous year. These results suggest that school-based anti-helminthic treatments are not reaching a substantial portion of the school-aged population in this part of Guatemala. Given that nearly 10% of adults were infected with a STH and only 23% reported receiving antihelminthic drugs, additional research should evaluate whether individuals age 18 years or older act as reservoirs for STH infection.

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COMPONENTS OF AN ANTIGEN CAPTURE ASSAY FOR THE DIAGNOSIS OF *ASCARIS* INFECTION

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Ascaris is the largest soil transmitted helminth known to cause human infection. It is estimated to affect one sixth of the world's population causing malnutrition, poor school performance and decreased response to vaccines. Current diagnosis of ascariasis is made using the Kato Katz method of microscopic examination of stool specimens. This method is time and labor intensive, technician-dependant and is not possible until one month into the infection when the life cycle is complete. Serological assays have been unreliable because of differences in host responses to Ascaris antigens. The development of an assay that could detect Ascaris

antigen in host bodily fluids in the early phase of infection would have wide applicability and utility. Immunoscreening of an A. suum infective larval stage 2 cDNA library was performed using sera from infected swine which identified ABA-1 as the immunodominant antigen at this stage. ABA-1 is an Ascaris antigen that has previously been described as a component of the Ascaris ES (excretory-secretory) protein which is produced and excreted by all stages of the parasite making it an excellent antigen target. ABA-1 protein is expressed by both A. suum which infects swine and A. lumbricoides which infects humans allowing a wider applicability of any successful assay. Recombinant ABA-1 fusion protein was produced from the ABA-1 containing clones using DNA isolated from the immunoscreening process. This DNA segment is homologous to that of the Ascarsis lumbricoides ABA-1. Two monoclonal anti-ABA-1 antibodies were generated by murine immunization and subsequent formation of immune B cell and murine myeloma hybridomas. Anti-ABA-1 activity has been confirmed by both ELISA and western blot assays to the recombinant ABA-1. Further characterization of the antibody epitopes should allow the development of an ELISA capture assay to aid in early diagnosis of Ascaris infections both in swine and humans.

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PREVALENCE AND SOCIODEMOGRAPHIC RISK FACTORS OF HELMINTH INFECTIONS AMONG ADULTS IN RURAL SOUTHWESTERN KENYA

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Rural southwestern Kenya suffers from high soil-transmitted helminth (STH) prevalence. Two studies of a rural village in the Nyanza Province demonstrated a pediatric STH prevalence that fell from 68% in 2007 to 44% in 2010, likely due to increased access to clean water, hygiene and sanitation training, and improved deworming coverage in the schools. We hypothesized that adults (≥18 years) have similarly high prevalence of STH infections and serve as reservoirs for re-infection of the children. We determined the prevalence of STH infection in the adults of this village and assessed socio-demographic factors associated with STH prevalence. We collected stool samples of 323 adults recruited at the local hospital and at four local schools. Basic demographic data were collected before stool sample screening. We used a highly sensitive sedimentation concentration technique for each sample. Concentrated specimens were stained with Dobell's iodine and examined for ova and parasites by light microscopy at 40x magnification. Adjusted prevalence ratios (PR) were estimated using multivariable regression. Prevalence of STH was 15.8% (51/323). Hookworm was most common (13.9% prevalence), followed by *Ascaria lumbricoides* (5.6%), and *Trichuris trichiura* (0.6%); no *Strongyloides stercoralis* infections were identified. Twelve participants (3.7%) had multiple STHs. Higher prevalence was associated with female sex (PR = 1.86; 95% CI: 0.97, 3.57) and age over 55 (1.94; 1.09, 3.45). Lower prevalence was associated with completion of some secondary school (0.17; 0.04, 0.71), occupation other than farming (0.30; 0.09, 0.95), and presence of any children living in the household (0.66; 0.34, 1.27). The STH prevalence of 16% found among adults was approximately one-third of that found among children, and may warrant regular de-worming of adults with risk factors for STH infection.

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COMPLETE CURE OF HUMAN HOOKWORM IN A HAMSTER MODEL USING A NOVEL APPROACH WITH CRY5B FROM *BACILLUS THURINGIENSIS*

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Two billion people in the world are infected with soil-transmitted helminths. The infections have devastating effects on human growth, nutrition, cognition, school attendance/performance, earnings, and pregnancy. Furthermore, the infections negatively affect HIV, malaria, tuberculosis, and modulate immune response, as reported previously. Currently the WHO recognizes 4 drugs (2 nicotinic acetylcholine receptor agonists, 2 benzimidazoles) for human treatment. The primary drug used for a variety of reasons is albendazole, and resistance to this drug is quickly emerging. Cry5B, a protein naturally produced in *Bacillus thuringiensis*, has been well characterized as an anthelmintic. Other Crystal family member proteins have been shown to be safe in humans, for example in transgenic corn, as a natural pesticide. This safety is thought to stem from multiple factors including the binding to an invertebrate-specific glycolipid receptor. However, there has never been a complete cure using Cry5B for *in vivo* experiments. The best, published result with Crystal protein (Cry5B) was an 89% hookworm reduction, as reported previously. Thus, we developed a novel method for using Cry5B as an anthelmentic. Consequently, we treated hamsters infected with human hookworm (*Ancylostoma ceylanicum*) with either Cry5B or control and achieved 100% cure in the Cry5B treated animals while the control treated animals had ~45 hookworms per intestine. This result constitutes the first time a 100% cure of intestinal worm burden has been achieved with a Crystal protein.

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FIRST-IN-HUMANS CLINICAL TRIAL OF THE *NA*-GST-1 HOOKWORM VACCINE IN BRAZILIAN ADULTS

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Necator americanus glutathione S-transferase-1 (*Na*-GST-1) is a 24-kDa protein that has peroxidase enzymatic activity that catalyzes the conjugation of reduced glutathione to a variety of electrophiles. *Na*-GST-1 belongs to a class of nematode GSTs that is characterized by diminished peroxidase activity relative to other GSTs but elevated binding capacity for heme and related products. It is produced by adult hookworms and is thought to play a role in detoxifying heme and other breakdown products of the hookworm blood digestion pathway. Vaccination of laboratory dogs and hamsters with recombinant GST-1 results in reduced hookworm fecal egg counts and adult worm burden following challenge with infective larvae. Recombinant *Na*-GST-1 was expressed in *Pichia pastoris* and formulated with Alhydrogel according to current Good Manufacturing Practice. A dose-escalation Phase 1 clinical trial is being conducted in which 102 healthy Brazilian adults will receive either *Na*-GST-1 or the hepatitis B vaccine. Volunteers vaccinated with *Na*-GST-1 will receive 1 of 3 different dose concentrations (10, 30 or 100 µg) in 1 of 2 different formulations (*Na*-GST-1/Alhydrogel or *Na*-GST-1/Alhydrogel to which 2.5 µg of the Toll-like receptor-4 agonist, glucopyranosyl lipid A [GLA-AF], has been added as a point-of-injection preparation). Participants will receive 3 intramuscular injections at 2-month intervals. The study is being conducted in 2 parts, first in healthy adults with no history of hookworm infection or exposure and living in the urban center of Belo Horizonte, and then in individuals living in a hookworm-endemic area of the Brazilian state of Minas Gerais. All participants are being screened for IgE antibodies to *Na*-GST-1 and will be excluded if positive due to the previous experience with IgE-related urticarial reactions being observed upon vaccination with the recombinant *Na*-ASP-2 hookworm vaccine. Monitoring for vaccine-related solicited and unsolicited adverse events will be performed following each vaccination and until 12 months after the final vaccination. Antigen-specific IgG antibody responses will be measured in vaccinated participants at multiple time points throughout the study. Preliminary safety and immunogenicity results will be presented.

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INSIGHTS INTO THE SEROEPIDEMIOLOGY OF TOXOCARIASIS IN JAMAICA

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Jamaica has seen a decline in the transmission of soil transmitted helminths due to improved living standards. However, the status of toxocariasis is not known and its public health significance has not been quantified. The aim of this study was to determine the seroprevalence of toxocariasis and to establish the age-prevalence profile for the infection in Jamaica. One thousand serum samples submitted to the Microbiology laboratory at the University of the West Indies in Kingston, Jamaica for diagnosis of dengue were assayed for IgG antibodies to toxocariasis using the Toxocara-CELISA (CeLLabs, Sydney, Australia). The prevalence of anti-Toxocara IgG was 19.8 % and males (11.2%) were significantly more likely to be exposed than females (8.2%) [$\beta 2 = 3.67$; p=0.046]. Furthermore, there was no association between exposure to Toxocara and area of residence (rural vs. urban) [$\beta 2 =0.835$; p = 0.409]. Prevalence of infection peaked in young adults and declined thereafter. This pattern may be reflective of reduced exposure to infective stages as persons get older and mirrors the pattern seen for some soil transmitted helminths. Transmission of Toxocara appears to be active in Jamaica and further studies to elucidate its clinical and public health significance are indicated.

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PATIENT AND SITE LEVEL PREDICTORS OF LOSS TO FOLLOW-UP AND MORTALITY IN PEDIATRIC AND ADOLESCENT PATIENTS IN NIGERIA

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As ART access for children increases, understanding factors that contribute to suboptimal outcomes is important. This study describes pediatric and adolescent outcomes in Nigeria and explores the impact of site characteristics on loss to follow-up and mortality. A retrospective chart review was conducted from 2007-2011. Data collected included age, weight for age (z score), ART regimen, visit dates and mortality. Loss to follow-up was defined as not having a visit within 90 days of the last missed visit. A cross-sectional survey was administered at sites providing care. Information gathered included use of guidelines, staffing, service integration, laboratory and pharmacy capacity. We performed descriptive analyses and examined associations with survival and loss to follow-up using Cox proportional hazards and logistic regression. 1,431 children were randomly sampled from 23 sites. Mean age at initiation was 64 months (SD 51.8). Severe immunosuppression was noted in 45% (644) at enrollment. Mean z score was -1.1 (SD 4.3). Median duration of follow-up was 24.8 months (IQR 29.8). Mean time from eligibility to ART initiation was 26.2 weeks (SD 44.4). Mortality during the period of follow-up was 4.4% (95% CI: 3.4%, 5.4%). Mortality within 90 days of ART initiation was 2.0% (95%CI: 1.3%, 2.7%). Loss to follow-up was 19.1% (95%CI: 17.1%, 21.1%) and associated with site type [primary vs secondary or tertiary hospitals (p=0.009)] but not with any site characteristics or patient level factors. Mortality was associated with degree of immunosuppression at presentation (p=0.03), z score (p< 0.004), and reported lack of full implementation of the 2010 Nigerian Pediatric Guidelines (p=0.04). In multivariate analysis moderate (p=0.024) and severe immunosuppression (p=0.037) were found to independently predict mortality. Treatment outcomes in the Nigerian program are encouraging. Interventions to ensure earlier access to ART, decrease loss to follow-up, and promote full implementation of the most recent guidelines are warranted.

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EXPOSED BUT UNINFECTED: DOES HIV EXPOSURE ALTER IMMUNE RESPONSES IN INFANTS?

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The introduction of the Prevention of Mother to Child Transmission (PMTCT) programmes has greatly reduced vertical transmission of HIV. In the developed countries, mother to child transmission (MTCT) has decreased to less than 1%. PMTCT coverage is fast increasing in sub Saharan Africa and in many areas the use of 1 dose of nevirapine has helped reduce MTCT of HIV to less than 12%. This has in turn led to an increasing number of infants who are exposed but not infected (EUs). In addition, with the improved care for HIV infected individuals, more women are reaching the child bearing age, further increasing the numbers of EUs being born. Increasingly, there is evidence to suggest that EUs may represent an immuno-compromised group with an increased probability of infections. Vulnerability to infections is not only due to increased environmental exposure to pathogens, but may also be due to induction of tolerance following *in utero* exposure to: several drugs, an activated mother's immune system that distorts the cytokine mileu and soluble antigens that may modify the infant's developing immune system and the way that they respond to antigens. Current evidence largely tackles potential disruptions to the T cell compartment and associated immune responses. In this study we sought to determine whether B cell/ humoral responses in the EU infants are different from HIV unexposed (healthy) controls. To do this, HIV EU infants of less than 18 months and age-matched healthy controls were enrolled and prospectively followed up until 24 months of age. Comprehensive B cell phenotypic analyses was done and data categorized into different age brackets of the HIV EU and control infants ie., 3, 6, 9, 12,15 and 18 months. At 18 months of age, frequencies of vaccine antigen-specific (measles, tetanus and diptheria) memory B cells were determined by ELISPOT assays. Antibody levels, and the associated isotype distributions and avidity were determined against the same vaccine antigens. Preliminary data will be discussed.

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ASSESSING ADHERENCE TO COTRIMOXAZOLE PROPHYLAXIS USING SELF-REPORT AND PILL COUNT AMONG HIV EXPOSED CHILDREN IN A SEMI-URBAN DISTRICT IN MALAWI

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Improved Prevention of Mother to Child Transmission (PMTCT) interventions have shown to reduce the risk of HIV infections among children. In HIV exposed children, prevention of HIV opportunistic infections is especially important given their susceptibility to severe illness and death. Cotrimoxazole prophylaxis given to these children until they stop breastfeeding and exclusion of HIV, has been an important intervention in preventing bacterial and other adverse health outcomes. HIV exposed children must adhere to cotrimoxazole prophylaxis to achieve optimal responses. Understanding adherence to cotrimoxazole prophylaxis in a population perceived to be 'healthy' is important in designing strategies for compliance. This study determines the rates and factors associated with adherence among HIV exposed children receiving cotrimoxazole prophylaxis. We conducted cross-sectional interviews with guardians of HIV exposed children participating in a large cohort study

of 500 HIV exposed and 500 non HIV exposed infants in a rural district in Malawi.Two approaches to assessing adherence were used: Self report and pill count done by the research team. A total of 315 interviews took place. Seventy percent (n=222) of the children as reported by guardians were adherent to cotrimoxazole prophylaxis for the past month before interviews. However, when pill count was used, only 59% (n=185) indeed adhered (p<0.001). Some of the reasons for non-adherence ranged from forgetting (25%), running out of the medication (30%) and being away from home (20%). For those who reported running out of medications as the reason for non-adherence, 20% was a result of sharing the drugs between the child and the parent. Factors associated with adherence included having a working mother [OR=3.04 (95% CI: 1.22, 7.56)] and having a mother with higher educational level [OR=0.22 (95% CI: 0.09,0.53)]. Self reported adherence to cotrimoxazole is high among HIV exposed children. However, objective measurements are necessary to ensure full compliance to prophylaxis if these children are to achieve optimal benefits from cotrimoxazole.

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STATUS OF HIV SEROLOGY AMONG PULMONARY TUBERCULOSIS PATIENTS: THE PATTERN OF PULMONARY TUBERCULOSIS AND THE CHARACTERISTICS OF PATIENTS - MARCH 2011

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Tuberculosis (TB) is a leading cause of death among people with HIV. In 2009, there was an estimate of 380,000 deaths due to TB among HIV patients. 78% of TB/HIV co-infection cases reside in sub-Saharan Africa, HIV prevalence is as high as 80% in some countries. Therefore, the prevalence of HIV infection in the study population of TB was determined. Clinical, laboratory and radiological presentation of TB were analyzed and compared between HIV sero-ve and HIV sero+ve patients. Observational case-finding hospital based study was done on 60 TB patients, performed in 3 hospitals in Sudan. Interview based questionnaires and medical records were used for data collection. Prevalence of HIV infected TB patients among the study population was 16.7%. The study revealed that 50% of the HIV+ve TB patients were younger than 30 years. There was no major sex difference between HIV+ve and -ve TB patients (P=0.905). However, males were predominant among the whole study population of TB patients. 60% of HIV+ve patients originated from the North, where as the origins of the HIV-ve patients were more or less equally distributed (P=0.012). Clinical presentations of HIV+ve and -ve TB patients were similar and the differences weren't statistically significant. When comparing the lab findings; +ve sputum smear was found more common among HIV+ve patients (70%vs.54%) and a +ve PPD test was also more common among HIV+ve patients (75%vs.50%). A clear CXR was the only statistically significant difference between the 2 groups, being more common in HIV+ve (40%vs.6%) (P=0.002). The study concluded that the prevalence found was low in comparison to most of the countries in the region but high in comparison to developed countries. The main differences between HIV+ve and -ve was a +ve sputum smear, +ve PPD test, and a clear CXR, all of which were more common in HIV+ve TB patients. Therefore, the TB/HIV programme should be strengthened; starting by knowing the exact incidence of TB/HIV among the population, implementing better diagnostic tools and more researches to be conducted on a larger scale.

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HIV/AIDS INFECTION AND HIGH-RISK BEHAVIORS IN A PARAGUAYAN MILITARY POPULATION

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We conducted a research study between July 2005 and January 2006 on 1,248 Paraguayan active duty military volunteers in order to evaluate HIV seroprevalence and sexual behavior risk factors. Participants provided a blood sample for HIV testing and answered an anonymous survey about sexual behavior and risk factors. This information, as well as demographic data (age, rank, gender), was evaluated using a multivariate analysis. The median age of first sexual intercourse was 16 years, with no statistically significant difference between military ranks (H, p>0.05). Only 14.8% (CI 95%: 12.9 to 16.9) of participants reported condom use with every sexual encounter. Military students used condoms the most. Participants older than 45 years, compared with younger participants, had a fourfold (AOR 4.29) increased risk of not using condoms. Males were less likely to use a condom, more likely to practice anal intercourse, and had more sexual partners than females. Officers and non-commissioned officers (NCOs) were found to have a twofold (as measured by adjusted odds ratio, AOR=1.96 and 2.24 respectively) increased risk of having more than two sexual partners in the last month compared with students. Likewise, male personnel had almost a fivefold (AOR=4.75) increased chance of having more than two sexual partners over the last month compared with females. Both officers and NCOs were twice as likely as students to practice anal intercourse. By asking different questions about sexuality, we observed that some participants who did not identify themselves as homosexual actually participated in intercourse with individuals of the same gender, oftentimes considering themselves heterosexual because they took the insertive role in anal sex with another male. Despite the high-risk behaviors reported by those surveyed, we found only five cases of HIV among the entire population (0.4%; CI 95%= 0.15-0.89). Although HIV seroprevalence in Paraguayan active duty personnel was low, future educational efforts should focus on the high-risk behaviors and high-risk groups identified in this study.

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MICROBIAL COMMUNITIES, GENITAL HEALTH AND HIV-PREVENTION POLICY

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The most severe generalized HIV epidemics developed in complex health environments in which multiple morbidities interact to accelerate HIV spread. Substantial evidence indicates that malaria, urogenital schistosomiasis, untreated STIs, other reproductive-tract infections, and nutritional deficiencies increase HIV transmission and acquisition by increasing viral load, genital viral burden, or causing genital lesions or inflammation. HIV-prevention protocols, however, do not include interventions to reduce these cofactors that enhance sexual and vertical HIV transmission. HIV in multi-burdened populations is poorly understood. STI-HIV trials are confounded by high prevalence of other genital lesions caused by schistosomiasis, staphylococci, or streptococci. Lessons from plant pathology and microbial communities of the gut should inform research on disturbance of genital mucosa and epithelia to understand sexual transmission of HIV. The protective ability of genital mucosa depends on microbial communities gravely disrupted by disease. Even with treatment for schistosomiasis or STIs, recovery of the genital environment in which sex occurs exhibits hysteresis - delayed restoration of integrity of

protective mucosa. As with STI trials, RCTs of schistosomiasis treatment for HIV prevention will face confounding from multiple genital morbidities. Also, ethical obstacles (because there are safe, proven, cost-effective treatments available for STIs and genital schistosomiasis) require that controls be treated, thus making it unlikely that statistically significant effects on HIV incidence can be detected, in spite of the beneficial effects on treated individuals. Insistence on confirmation of treatment for cofactors (STIs, schistosomiasis) with an unachievable 'crucial experiment' for complex health problems, such as HIV in sub-Saharan Africa, is incorrect epidemiology and bad public health policy. Lack of full scientific certainty should not be used as a reason for postponing cost-effective measures to prevent threat of irreversible damage.

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EVALUATION OF A PRO-ACTIVE STRATEGY FOR MANAGING TB-HIV CO-INFECTION IN A UK TERTIARY CARE SETTING

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Management of TB-HIV co-infection is complicated by multiple interactions between diseases and therapies. We developed and evaluated a five-element strategy to: (i) treat co-infected patients in a single co-infection clinic, (ii) maximize use of first-line drugs, (iii) delay anti-retroviral therapy (ART) until 2 months post-TB treatment except in severe immunosuppression, (iv) commence efavirenz at 600mg daily with therapeutic drug monitoring (TDM), and (v) target treatment completion. Prospective cohort review over 5.5 years in a UK tertiary referral center. Fifty-six HIV-positive patients treated for TB were followed-up for a median 30 months. Main outcome measures were treatment completion, adverse events, IRIS, immunological and virological parameters and TDM for efavirenz. TB frequently presented with low CD4 counts (69% <200 cells/μL) and multiple, concomitant opportunistic infections. 15/56 cases (27%) occurred after ART commencement as 'unmasking IRIS'. First-line TB therapy and ART was used in 93 and 91% of cases respectively. Adverse events were common (55%), but caused no treatment interruptions. Treatment completion rates were 88% (49/56); four patients were lost to local follow-up and three (5.4%) died during treatment; no deaths were TB-related. Efavirenz TDM in patients receiving rifampicin showed very wide inter-individual variation (580 to 15,325 ng/L) but standard doses (600mg daily) achieved or exceeded therapeutic levels in 25/28 (89%). In conclusion, this study supports combined management for TB-HIV co-infected patients. Other opportunistic infections are common, but delaying ART to 2 months post-TB treatment did not seem to result in poor clinical outcomes. Although Efavirenz 600mg daily usually achieved satisfactory levels, TDM is recommended.

1138

HIV PATIENT WITH MUCOSAL LEISHMANIASIS TREATED WITH MILTEFOSINE IN COLOMBIA: A CASE REPORT

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Co-infection with *Leishmania* and Human Immunodeficiency Virus (HIV) has been reported in 34 countries around the world, mainly in southern Europe, where most visceral leishmaniasis cases. A few reports of cutaneous manifestations of leishmaniasis in patients infected with HIV have been reported. These groups of patients depict atypical manifestations of the disease and are at higher risk of leishmaniasis dissemination due to depletion of both humoral and cellular response of the organism. We report the case of a 27 year-old male patient, resident of Cáceres, Antioquia, in northeast Colombia, who was diagnosed with HIV with a history of cutaneous leishmaniasis years ago and then presented with Leishmaniasis in nasal mucosa. He was treated with miltefosine and after finishing treatment relapsed, needing to be treated with systemic pentavalent antimonials. This patient had a low CD4 count during the treatment and a bad compliance with the HAART therapy, which can be the cause of his relapse. There is a clear synergy between the two diseases, the unpredictable course and the challenge it poses to the treating physician. The atypical presentations of disease in *Leishmania* and HIV co-infection force to make a comprehensive approach to the diagnostic possibilities to reach the final pathology and to provide adequate and soon management. This is not only important to create more awareness within the scientific community, but to reinforce the fact that we need to count with a better therapeutic arsenal to treat tropical neglected diseases, since co-infection is increasing all around the world.

1139

KNOWLEDGE, ATTITUDE AND PRACTICE OF MALARIA PREVENTIVE MEASURES AMONG HIV POSITIVE AND HIV NEGATIVE PREGNANT WOMEN IN SOUTHWESTERN NIGERIA

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The pregnant woman is more prone to malaria than her non-pregnant counterpart with adverse pregnancy outcomes. HIV infection further increases the susceptibility of the pregnant woman to malaria and its consequences. Between July 2009 and August 2010, 1742 pregnant women [HIV +ve -497 (28.5%); HIV -ve 1245 (71.5%)] were enrolled at first antenatal clinic attendance in a tertiary hospital in southwest Nigeria. A self/investigator-administered questionnaire was used to collect information. Haematocrit and presence of malaria parasitemia were evaluated. Chi-squared was used to investigate association between categorical variables and ANOVA for continuous variables. Level of significance was set at β= <0.05. The mean age (±sd) was similar among the two groups of pregnant women. Prevalence of malaria parasitemia [12.7% versus 6.5%; β=<0.0001] and geometric mean parasite density [24,940 versus 8,550; β= 0.144] was higher among HIV +ve women. Mean haematocrit [30.8%±4.5 versus 34.25%±4.02; β=<0.0001] was significantly lower among HIV +ve pregnant women compared to HIV -ve women. Significantly more HIV +ve than HIV -ve women did not think that malaria was preventable [β= <0.0001]. Less than 12% of the study population [HIV -ve women more than HIV +ve women [10.6% versus 1.1%; β= <0. 0001] knew that malaria parasite was the causative agent for malaria while a larger proportion knew that mosquitoes were the vectors that transmit malaria [HIV -ve versus > HIV +ve; β= <0. 0001]. HIV -ve women were also significantly better informed about anti-vector measures and malaria chemoprevention [IPT, proguanil]. Although significantly more HIV -ve women had heard about ITN and were willing to use ITN (β= <0. 0001]. ITN ownership was similar in both groups of pregnant women (44.2% -HIV +ve; 40.2% -HIV -ve; β= <0.154). 24.9% and 20.1% (β= <0.098) of HIV +ve and HIV -ve women claimed to have slept under an ITN the night before the survey. In conclusion, prevalence of malaria parasitemia is significantly higher among HIV +ve than HIV -ve women. Inadequate knowledge and use of malaria preventive measures among pregnant women and especially HIV +ve pregnant women recorded in this study is a matter of concern. There is a need for a robust information, education and communication programme on malaria for all pregnant women in southwest Nigeria.

1140

PREVALENCE OF HIV INFECTION IN RELATION TO DEMOGRAPHIC/OBSTETRICS DATA AND MALARIA INFECTION AMONG PREGNANT WOMEN IN CENTRAL NIGERIA

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HIV infection has been found to increase the incidence and severity of clinical malaria. In non pregnant adults, it has been found to roughly double the risk of malaria parasitaemia and clinical malaria. A total of 200 pregnant women attending ante natal clinic in Jos University Teaching Hospital were sampled after obtaining informed consent. In relation to age, HIV infection was more prevalent among pregnant women aged between 12 and 19 years (64%, 95% C.I:50.7 - 77.0%) followed by women aged between 20 and 29 years (62.2%, 95% C.I: 52.3-72.1%). HIV was least common among the ≥ 40 year olds (40.0%, 95% C.I: 30 - 83.0%). There was however, no statistical difference (P>0.05) in HIV infection among the different age groups (Cal β = 0.8134 <Tab β 0.05 df_3 = 7.815). On parity, HIV was more prevalent among the primiparous pregnant women (72.6%, 95% C.I: 62.4 - 82.8%) followed by those who have had two children (59.3%, 95% C.I: 46.2-72.4%). The least prevalence was recorded among those who have six or more children (42.9%, 95% C.I: 6.2-79.6%). There was also no significant difference among the different parity levels (Cal β = 3.7388 < Tab β 0.05 df_3 = 7.815). In relation to gestational level, HIV infection occurred more among those in their 3rd trimester (66.7%, 95% C.I: 48.9 - 84.5%) followed by those in their 2nsd trimester (63.2%, 95% C.I: 54.6 - 71.8%). The least HIV prevalence was among those in their 1st trimester (50.8%, 95% C.I: 38.0-63.6%). There was no statistical difference (P>0.05) between the various gestational levels (Cal β = 1.2132 < Tab $\beta_{0.05}$ df_2 = 5.991). On coinfection of HIV and malaria, 26/41 (63.4%, 95% C.I: 48.7-78.1) of those infected with malaria also had HIV infection, while 94/159 non malaria patients had HIV infection (59.1%, 95% C.I: 51.5-66.7%). There was however, no statistical difference (P>0.05) in HIV prevalence between those infected with malarial and those who were not (cal β = 0.0617<Tab β 0.05 df_1 = 3.841).The public health significance of these findings are discussed.

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ANTIRETROVIRALS VS. STANDARD ANTIMALARIALS/ CO-TRIMOXAZOLE AS MALARIA CONTROL DRUGS IN HIV PATIENTS

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Previous studies found an increase in malaria among HIV patients in some regions. Also, malaria has been implicated as an enhancer of HIV replication and infectivity. One hundred HIV positive adults were recruited in Lagos, Nigeria. Drug history was ascertained by oral interview and review of clinic records. Blood samples were collected and laboratory tests carried out to obtain blood groups, Genotype, Malaria parasitaemia, CD4 counts, and Viral load. Malaria parasitaemia was significantly less among those on ARVs (Lamivudine, Zidovudine, Nevirapine) compared with those not on ARVs; 9.65 vs 13.3%(p=0.006). Patients placed on single course Artemisinin Combination Therapy, Sulphadoxine/pyrimethamine and Chloroquine within the last 28 days were without parasitaemia. Though 60% of patients on Co-trimoxazole had malaria parasitaemia this drug appeared to cause increase in CD4 values as average malaria parasite density declined. Our findings suggest that the criteria which govern use of these ARVs and antimalarials in malaria endemic regions need to be reviewed, promoting an earlier commencement of ARVs.

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TARGETING NEGLECTED DISEASES BASED ON RATIONAL APPROACH DESIGN; PROOF OF CONCEPT: NOVEL PEPTIDES FOR INHIBITING LEISHMANIASIS

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For over 20 years, we have developed short peptide inhibitors of protein-protein interactions between signaling enzymes, such as protein kinase C (PKC), and its scaffold protein, receptor for activated C-kinase (RACK). These short bioactive peptides are highly selective and effective in several animal models of human diseases. Some of these peptides were tested in humans and were shown to be safe. RACK interacts with and regulates multiple signaling enzymes that have key cellular functions. The RACK ortholog in *Leishmania*, called LACK, is not well characterized but is functionally critical. Parasites in which LACK was knocked out are not viable, and parasites that express low levels of LACK fail to parasitize even immune-compromised mice. Because of its homology to RACK, we assumed that LACK also interacts with multiple signaling enzymes in the parasite and might be a key scaffolding protein involved in essential signaling processes. Furthermore, LACK is found in both amastigotes and promastigotes of *Leishmania*. Therefore, we predicted that LACK is a good drug target, and we developed novel peptides aimed at inhibiting LACK interactions with LACK-binding proteins. Peptides were developed based on a sequence homology search and structural studies and were conjugated to TAT-derived peptide for drug delivery. When used to treat L. amazonensis promastigote cultures for 24 hours, some peptides resulted in growth inhibition with IC_{50} of approximately 10 μM. Furthermore, these peptides inhibited infection of macrophages by L. amazonensis promastigotes. The peptides are non-toxic to macrophages. Therefore, without any knowledge on partner proteins of LACK, we were able to design presumed inhibitors of LACK's function and affect the parasite's viability. Our method is likely applicable to design other anti-parasitic drugs.

1143

POLYMERASE CHAIN REACTION DETECTION OF *TRYPANOSOMA CRUZI* IN SUGAR GLIDER (*PETAURUS BREVICEPS*), HEDGEHOG (*ATELERIX ALBIVENTRIS*) AND CHIMPANZEE (*PAN TROGLODYTES*) USING ARCHIVED TISSUES

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We report a PCR based method to detect *Trypanosoma cruzi* organisms in formalin fixed paraffin embedded blocks from 3 sugar gliders, 2 hedgehogs and 1 chimpanzee which were previously diagnosed as *T. cruzi* positive by histopathology. Heart samples from animals referred to the Zoo/Exotic Pathology Service (West Sacramento, CA) were fixed in formalin, and embedded in paraffin blocks using conventional methods. For the PCR assays samples were obtained from the paraffin wax blocks using a 3-mm tissue punch (Acuderm. Inc, Fort Lauderdale, FL) and de-paraffinized overnight in xylene. DNA was extracted using the DNeasy kit (Qiagen, Valencia, CA) according to the manufacturer's specifications. PCR was carried out using primers TCZ1 and TCZ2 and platinum *Taq*

polymerase (Invitrogen) under touchdown conditions. The PCR products were stained with ethidium bromide, visualized under UV illumination and photo-documented. Although *T. cruzi* has previously been reported in chimpanzees and cynomolgus monkeys, this is the first time this organism has been detected in sugar gliders and hedgehogs. In one hedgehog no *T. cruzi* organisms were found by histopathology but cardiac tissues were positive by the PCR method, demonstrating the higher sensitivity of the PCR method. Successful use of DNA from formalin-fixed, paraffin-embedded blocks is important because most pathology laboratories routinely archive tissue blocks. These archived paraffin embedded tissues can be used for further studies on the prevalence of this disease..

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INNOVATIVE SERUM-FREE MEDIUM FOR *IN VITRO* CULTIVATION OF PROMASTIGOTE FORMS OF *LEISHMANIA* SPECIES

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We described a comparatively simple medium formula (CML) using common, available and reasonably priced ingredients that could be used in place of medium that requires calf serum enhancement for cultivation of *Leishmania* promastigote forms. This medium equivalently supported the growth of parasites at rates comparable with those obtained with serum supplemented RPMI-1640 medium. *Leishmania* promastigotes reproduced in CML exhibited moderate to high infectivity capacities when tested against J774 macrophage cell line. No significant difference was noted between *Leishmania* strains cultivated in the newly modified medium and those grown in RPMI-1640 medium in their cells infectivity and replication potentials. The use of new CML can easily take the place of other biphasic or liquid media because of its easy preparation and instantaneous use, reasonable price, availability of ingredients, and its long shelf life, which is 30-45 days. The fact that this medium is similar to other culture media as far as durability and quantity of produced parasites might give it an advantage over the other currently used media.

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IDENTIFICATION OF INHIBITORS OF *TRYPANOSOMA CRUZI* INTRACELLULAR AMASTIGOTE REPLICATION BY FLUORESCENCE-BASED *IN VITRO* IMAGING ASSAYS

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Chagas disease (CD), caused by the protozoan parasite *Trypanosoma cruzi* causes over 15 million deaths per year and Chagas Heart Disease is the leading cause of infectious myocarditis in the world. CD, endemic to South and Central America, is designated a neglected tropical disease by the World Health Organisation and the National Institute of Health. Treatment of CD is limited to the nitroheterocyclic drugs benznidazole and nifurtimox that cause severe side effects and show very poor efficacy against the second, chronic phase of the disease. The search for new, more effective and less toxic drugs is a continuing development in CD research. To discover new active compounds against the parasite we have developed an imaging- based assay to enumerate *T.cruzi* intracellular amastigotes following exposure of *T.cruzi* infected 3T3 host cells to compounds or known control drugs. The assay technology utilises nuclear and cytoplasmic markers to fluorescently stain both the parasite and host cells, detected with a High Content imaging system and analysed with a developed script. With the use of 3T3 fibroblasts as the host cell, of which growth is not effected by *T. cruzi* early stage infection (as reported previously), host cell survival can also be estimated as a primary determination of compound mediated cytotoxicity in the one assay. This assay was utilised to determine the activity of a compound library of FDA approved and biologically active compounds. The most successful chemical candidates for further biological evaluation will be outlined. The development of a downstream assay relevant to human infection to assess compound activity against *T.cruzi* amastigotes internalised in primary human heart fibroblasts, along with a trypomastigote imaging-based assay will be presented. Although trypomastigotes are not the most clinically relevant form in consideration of the disease state, activity against this motile infective form would be favourable. These assays will be utilised to further profile the activity of selected compounds.

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USE OF 16S RRNA GENE UNIVERSAL PRIMER PAIR AS A PCR AMPLIFICATION POSITIVE CONTROL DURING THE DIAGNOSIS OF *TRYPANOSOMA CRUZI* IN ARCHIVED TISSUES FROM 4 MAMMALIAN SPECIES

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Trypanosoma cruzi infects a variety of mammalian species including humans, pets, and wild animals. To develop a PCR-based method for the detection of *T. cruzi* we tested archived tissues from chimpanzees, cynomolgus monkeys, hedgehogs and sugar gliders and characterized 16S rRNA as a DNA positive control that would make it possible to distinguish between PCR failure and truly negative results. The traditionally used PCR primer sets like β-globin and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) could not be used due to the species wide genetic differences. The16S rRNA primer set successfully amplified DNA from primates, marsupials and hedgehogs suggesting it is a potential universal DNA positive control primer for PCR-based test for *T. cruzi*. The priming sites of this primer set are sufficiently conserved among mammalian species and this primer set has been used before for species identification among vertebrates.The sequence of the 16S rRNA is: L2513 Forward 5'-GCCTGTTTACCAAAAACATCAC-3' and H2714 Reverse 5'-CTCCATAGGGTCTTCTCGTCTT-3'

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IDENTIFICATION OF NOVEL ANTI-LEISHMANIAL CHEMOTYPES THROUGH A SYSTEMATIC SCREENING OF KINASE INHIBITOR LIBRARY

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Leishmaniasis is caused by different species of protozoan parasites and is transmitted by the bite of an infected sandfly. Leishmaniasis is endemic in 88 countries with 350 million people at risk. Current therapies are limited by high cost, toxicity, compliance, parenteral administration, and cold storage. We have identified lead chemotypes with activity against leishmania through a systematic screening of 34 diverse structural classes from a large library of kinase inhibitors. The compounds were screened for *in vitro* activity against *Leishmania major, L. donovani* (promastigotes and axenic amastigotes) and for cytotoxicity in a mouse fibroblast cell line (L929). After four rounds of screening, with analog selection based on activity and cytotoxicity, two structurally distinct compound series were identified with IC_{50} values ranging from 30 nM to >5 μM. The top compounds were further evaluated in an intracellular assay. Mouse macrophage cells, infected with either *L. major* or *L. donovani* strains with an integrated luciferase reporter, were treated with compounds for 72 h and IC_{50} for parasite inhibition was determined by luminescence. Metabolic stability in the presence of mouse microsomes and cellular permeability in an MDR1-MDCK cell line was also assessed. Based on activity against intracellular parasites, metabolic stability and permeability assessments, four compounds were evaluated in a mouse model of *L. donovani* at 2 independent sites. Efficacy was calculated based on

reduction in liver infection as compared to untreated infected animals. Reduction in liver parasites of up to 43% was observed. Based on these results, we have initiated a SAR program to synthesize new analogs, targeting maintained or improved potency, but with improved ADME properties.

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REAL-TIME *LEISHMANIA* GENUS MASTER MIX: A STABILITY AND PLATFORM COMPARABILITY STUDY

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Leishmaniasis is a disease cause by the bite of a leishmaina infected sand fly of the species, *Phlebotomus* or *Lutzomyia*. Leishmaniasis is endemic in 88 countries and 10-15 million individuals are infected worldwide. Sand flies are native to the tropical and subtropical regions[1] where many American and allied soldiers are stationed. Therefore, it is necessary to have a molecular detection method for conducting *Leishmania* surveillance in the field. The Walter Reed Army Institute of Research has produced a "bulk" lot of *Leishmania* Genus real-time PCR master mix using a wet chemistry that has been validated in accordance with College of American Pathologists standards for use on the SmartCycler® instrument (Cepheid; Sunnyvale, CA). This bulk master mix was divided into 450µL aliquots and stored at -20°C. A verification study using a Limit of Detection (LoD) was performed to compare the bulk master mix to a newly prepared lot of master mix, and these results served as the T_0 time point for the stability study. Overall, results from the stability study indicate that the bulk *Leishmania* Genus master mix is stable when stored at -20°C. However, the Joint Biological Agent Identification and Detection System (JBAIDS, ITI; Salt Lake City, UT) is the Program of Record PCR platform utilized by the armed forces. Consequently, a LoD study was performed using the bulk lot of master mix to determine whether this wet chemistry would be compatible with the JBAIDS instrument. The following serial dilutions (400pg/µL, 40pg/µL, 4pg/µL, and 0.4pg/µL of *L. tropica* EP 139) produced positive results with average crossing point (Cp) values, which were three Cp values apart. A sensitivity and a limited specificity study were performed using both the SmartCycler and JBAIDS platforms, in which both platforms produced comparable results. Based on these results, the bulk lot of *Leishmania* Genus master mix is compatible with the JBAIDS. We foresee this assay to be used by armed forces in the field, which will have a significant effect on vector surveillance.

1149

CLONING, EXPRESSION AND PURIFICATION OF *LEISHMANIA DONOVANI* ANTIGEN FOR THE DIAGNOSIS OF VISCERAL LEISHMANIASIS

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Microscopic observation of amastigotes in splenic or bone marrow biopsies and serology including rK39 dipstick test are tools commonly used for diagnosis of visceral leishmaniasis (VL). However, bone marrow and splenic aspiration are painful and risky procedures and serology with rK39 dipstick can yield false positive responses in 20-32% of endemic healthy individuals. Identification of additional *Leishmania donovani* antigens could increase the specificity of noninvasive serologic testing for active VL. Therefore, we screened promastigotes soluble proteins using western blotting with a series of serum specimens from patients with acute VL. Western blots revealed a protein of molecular weight 70kDa (BHUP1), recognized by sera from VL patients but not healthy controls. Mass spectrometry of the gel-purified protein revealed the antigen as *L. donovani* HSP70. The full length *hsp70* gene of 1959 nucleotides was determined, cloned and expressed as a His-tagged fusion protein, purified, and retested. Antibody against this protein were detected in more than 96% of serum samples from patient with VL but not detected in sera from the endemic and non endemic control persons. Cross-reactive responses of sera from subjects with different disease like malaria and tuberculosis revealed the BHUP1 antigen test is highly sensitive for VL, but specificity was too low to differentiate from other infectious diseases

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INVESTIGATING TRYPANOTHIONE AS A BIOMARKER FOR TRYPANOSOMAL INFECTION

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Trypanosomatids are protozoan parasites that cause three neglected human diseases: Chagas disease in Latin America, human African trypanosomaisis, and leishmaniasis in 38 countries. We are developing diagnostic tools based on the detection of the parasite-specific small molecule trypanothione (TSH_2). Trypanosomatids use TSH_2 to modulate their redox metabolism in conjunction with trypanothione reductase, which is orthogonal to the system of glutathione and glutathione reductase used in their mammalian hosts. We hypothesize that TSH_2 can be used as a biomarker in the development of diagnostics for the early detection of trypanosomatid diseases. A sensitive and, inexpensive point-of-care (POC) diagnostic for early detection would prove invaluable in the treatment and control of these devastating parasitic diseases. In our preliminary studies towards a POC approach, we have shown that the bisarsenical probe fluorescent arsenical helix (FlAsH) binder ethane dithiol (EDT) adduct can be used to detect the parasite metabolite TSH_2. Our work in buffer solution showed that FlAsH-EDT_2 has 0.4% of the fluorescence of the FlAsH-$(TSH_2)_2$ conjugate. We are able to detect the metabolite in extracts from rat serum. Our second generation of molecular probes outperforms FlAsH for this application. These probes have a lower limit of detection and faster rate of detection. Most encouraging was our observation that the metabolite is detectable in serum extracts using a simple handheld UV lamp. That detection indicates that arsenical probes could be valuable tools for the development of a low technology, inexpensive, and rapid POC diagnostic for trypanosomatid diseases. We are also exploring the detection of TSH_2 using more conventional antibody detection for seamless integration into existing clinical infrastructure. We will describe proof of concept experiments for the detection of TSH_2 from cultured *Leishmania* and *Trypanosoma cruzi* using these complementary approaches.

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EVALUATION OF THE ACTIVITY OF THE COMBINATION OF AZITHROMYCIN PLUS FLUCONAZOLE AGAINST *LEISHMANIA (V) BRAZILIENSIS* AND *LEISHMANIA (L) AMAZONENSIS* IN GOLDEN HAMSTERS

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The exploration of new oral therapeutic options for the treatment of American Cutaneous Leishmaniasis is a research priority with a potentially significant impact in patient management. We have demonstrated the activity of azithromycin PO against *Leishmania braziliensis* although inferior to meglumine antimoniate, whereas no activity on *L. amazonensis* was detected. In the current study, golden hamsters were infected on hind foot with $5x10^5$ metacyclic promastigotes of *L. braziliensis* or *L. amazonensis*. Five groups were established: azithromycin PO 400 mg/kg/day (A); fluconazole PO 80 mg/kg/day (F); azithromycin PO plus fluconazole PO

20 mg/kg/day (F/A); meglumine antimoniate IM 60 mg/kg/day (G) and untreated animals (C). The treatment was administered immediately after infection and for 28 days. Size of lesions was determined weekly. A week after finishing treatment, animals were sacrificed. Parasite load in skin was measured by limiting dilution assay and cultures of skin, lymph node and spleen were made. In the *L. braziliensis* model, there were no significant difference in the progression of size of lesions in G compared to F/A, except in week 5, one week after treatments were completed, when G performed significantly better. Lesion size was smaller in G vs. C from week 2 until the end of the study, and in G vs. A in weeks 4 and 5. Similarly, F/A showed smaller lesion size since week 3 comparing to C. There were no significant differences in paired comparisons of other groups. In the limiting dilution assay, G, F/A and A showed no differences between them, while they showed less parasite burden than F and C. There was no difference between F and C. Cultures of infected foot and lymph node were positive in almost all animals, while the positivity of spleen were 50% in C, 40% in F, 10% in F/A and 0% in G and A. In the *L. amazonensis* model, only G showed activity. In conclusion, fluconazole alone has no activity for *L. braziliensis* and the combination fluconazole/azithromycin appears to have no additional effect than azithromycin monotherapy.

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HIGHLY ACTIVE ANTI-*LEISHMANIA* H2 MOLECULE DESIGNED *IN SILICO* TO INHIBIT PTR1 AND DHFR

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There is an urgent need for finding better and more efficient treatments for leishmaniasis, which should be less toxic, oral administrated, price affordable, and mainly and most important: endowed with good efficacy. Sixteen molecules were synthesized after extensive *in silico* design and selection by their theoretical ability to inhibit PTR1 (Pteridine Reductase-1) and DHFR (Dihydrofolato-reductase). Then the molecules were tested in *in vitro* culture assay of *Leishmania mexicana* M379 strain. The therapeutic response of the newly synthesized compounds was studied in a mice model of heavy infection by *L. mexicana*. The selected compounds were orally administrated to infected mice. The therapeutic effect was evaluated following lession size, dermatosis, mice activity, mortality, longevity, and parasite presence in blood by using PCR with JW11 and JW12 primers. One molecule called H2 and its acetylated precursor, H2A, were selected due to their good ability to achieve a quick and effective *Leishmania* growth inhibition by death induction of the parasite since the first 30 min at a dose between 1-10 µg/mL. The compounds were found to have therapeutic effect in eliminating parasites from the skin and blood, stopping lesion size increase ($P < 0.05$, Student's t Test) reducing mice mortality and prolonging mice life ($P < 0.05$, Chi Square Tests) at doses of 0.1 and 1 mg/mL given *at libitum* in drinking water during 3 days and up to two weeks. No adverse effects were noticed at these doses. The complete elimination of parasites could not be reached yet, since PCR that became negative during treatment, detected parasites in blood two months after treatment end. In conclusion, compounds H2 and H2A are highly active in anti-*Leishmania* therapy. The complete eradication of the parasite has not been achieved yet, but there is still work on this matter to be done. Extending treatment length, making compound combinations or preparing soluble compound formulations could increase the erradication rate of the parasite.

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FUNCTIONAL AND PHENOTYPIC PROFILE OF CD4+ AND CD8+ T CELLS IN *TRYPANOSOMA CRUZI*-INFECTED CHILDREN SUBJECTED TO TREATMENT WITH BENZNIDAZOLE

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In this study, we sought to gain a clearer understanding of the relationship between parasite persistence and the maintenance of *Trypanosoma cruzi*-specific T cells by examining the functional profile of *T. cruzi* antigen-responsive T cells and T cell phenotypes prior and after treatment with benznidazole (BZ) of 25 *T. cruzi*-infected children in the indeterminate phase of Chagas disease. The evaluation of the functional status of parasite-specific T cells before treatment with BZ showed that the majority of the patients display *T. cruzi*-antigen responsive $CD4^+$ producing both IFN-γ and TNF-α T cells, indicating polyfunctionality of the parasite-specific T cell compartment. Increased frequencies of terminally differentiated effector ($CD45RA^+CCR7^-CD62L^-$) and effector memory ($CD45RA^-CCR7^-CD62L^-$) phenotype $CD4^+$ and $CD8^+$ T cells were also observed in *T. cruzi*-infected children compared with uninfected controls. Within 8 months of treatment with BZ, the levels of total early-differentiated memory ($CD45RA^-CD27^+CD28^+$) T cells increased while the levels of fully differentiated memory T cells decreased ($CD45RA^-CD27^-CD28^-$) in most patients. The expression of $CD127^+$ (IL-7 receptor) on $CD4^+$ and $CD8^+$ memory T cells was also increased following treatment. These changes in the phenotype of the overall T cell compartment were associated with a significant reduction in *T. cruzi*-specific antibodies, as assessed by conventional ELISA assays. Our results show a significant impact on the T cell compartment early after treatment with BZ which might be indicative of a reduction in parasite load after treatment of children in the indeterminate phase of Chagas disease. The high proportion of children displaying polyfunctional T cell responses specific for *T. cruzi* is compatible with a more competent immune status in these subjects compared with our former findings in adult *T. cruzi*-infected individuals, supporting our hypothesis that very long term chronic infection eventually results in T cell exhaustion.

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CLONING, EXPRESSION AND IMMUNOLOCALIZATION OF A *TRYPANOSOMA BRUCEI* CONSERVED HYPOTHETICAL PROTEIN

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The identification and cellular localization of novel proteins in *Trypanosoma brucei* will provide valuable information for the identification of new drug, vaccine or diagnostic targets. In this study, an ORF coding for a hypothetical protein, designated tmp10, was identified using bioinformatics tools predicting it to be a surface protein (Tmp10). Tmp10 was successfully cloned from *T. brucei brucei* GVR35, expressed and purified as a partial-length, His-tagged recombinant protein (rTmp10) in *E. coli* BL21 (DE3). When probed with polyclonal rabbit anti-rTmp10, a Western blot on T. brucei brucei whole cell lysate indicated that Tmp10 is a ~25kDa protein, whereas analysis by indirect immunofluorescence microscopy indicated that the protein might be localized on the surface of the parasite. Furthermore, when cultured *in vitro* with anti-rTmp10 serum, inhibition of parasite growth was observed and this inhibitory effect was dependent on both serum concentration and time of incubation. These results suggest that Tmp10 is a novel surface protein expressed endogenously by *T. brucei brucei*, and antibodies against it could provide

protective immunity against trypanosomiasis. The results also illustrate the merits of mining genome databases using bioinformatics tools as a cost-effective way to facilitate the identification of novel proteins.

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IMMUNE STATUS IN PATIENTS WITH CUTANEOUS LEISHMANIASIS WITH THERAPEUTIC FAILURE

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Currently little is described about the immunological aspects of patients with cutaneous leishmaniasis (CL) with resistance to the main therapeutic drug (Pentavalent antimonials). The study evaluated and compared the immune status the patients with therapeutic failure ("Resistant") and patients who responded successfully to treatment ("Sensitive"). Patients with CL living in Bolivia, an area highly endemic for *Leishmania* sp. were enrolled into the Resistant and Sensitive groups mentioned about. Measurement parameters of the immune response were: CD4+ and CD8+ T cells, production of IFN-γ and IL-13 as markers of Th1 and Th2 response respectively, by peripherical blood mononuclear cells (PBMCs) stimulated with Antigen soluble leishmania (SLA). PCR analysis was performed to typify sp. linages the leishmania parasites, isolated from skin lesions of patients Resistant: The result show that: CD4+ and CD8+ T cells were below normal values in both study groups, these values of CD4+ and CD8+ T cells in both groups showed no statistically significant differences. Resistant patients developed a strong IFN-γ response to SLA than Sensitive patients: Production of IL-13 remained low and similar in both groups. The characterization of strains isolated from patients Resistant identified to *L. brasiliensis* and *L. guayanensis*. These results show that: i) the specific immune response of resistant and sensitive patients is polarized toward TH1 ii) Values of CD4+, CD8+ T cells indicate an immunodeficiency in both study groups iii) Studies of Molecular Biology, showed predominance of *L. brasiliensis* in most clinical cases iv) The results do not fully explain the treatment failure in Resistant patients, hypothetically we are thinking about parasite-related factors (resistance genes).

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EVALUATION OF IMMUNOLOGICAL MARKERS IN PERUVIAN SUBJECTS ASSOCIATED WITH DIFFERENTIAL OUTCOMES OF *LEISHMANIA* INFECTION

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Many studies support the notion that cell-mediated immunity involving a Th1 cytokine pattern is important for a protective immune response against Leishmaniasis. In this study, we investigated the Leishmania-specific lymphocyte response and cytokine production in different clinical manifestations of American Tegumentary Leishmaniasis (ATL). Our aim was to assess immunological factors associated with the clinical outcome of ATL. PBMCs from 20 active cutaneous leishmaniasis patients (ACL), 17 cured cutaneous leishmaniasis patients (CCL), 8 asymptomatic individuals (ASY) and 14 endemic negative controls (ENC) were stimulated with total soluble Leishmania antigen (TSLA). Lymphocyte immunophenotyping for T-helper (TCD4), T-cytotoxic (TCD8), B (CD19) and NK (CD16/56) cell subsets were determined by flow cytometry in whole blood and stimulated PBMCs. In addition, Th1/Th2/Th17 cytokines were measured in culture supernatants. Asymptomatic individuals were defined as those whose IFN-γ production was above the cut-off value (Mean of 9 non-endemic negative controls + 2SD = 13.08 pg/ml). Only ASY individuals showed significantly higher T cell population after stimulation when compared with ENC ($p<0.05$). No statistically significant differences were found in TCD4 and TCD8 cells in stimulated PBMCs among the evaluated groups. Interestingly NK cells showed higher number in ACL and CCL in both whole blood and stimulated PBMCs, when compare with ENC ($p<0.05$). Statistically significant higher values were found for IFN-γ levels in ASY, CCL and ACL; for TNF-α and IL-10 in ACL; for IL-17 and IL-2 in ASY ($p<0.05$), when those levels were compared with ENC. For IL-2, the opposite was observed in ACL who showed lower production in relation to ENC ($p<0.05$). Given that TCD8 and TCD4 proportions were not statistically different among the evaluated infected groups, while IFN-γ levels were increased according to clinical status (ACL>CCL>ASY), we suggest that NK cells could be an important IFN-γ source. Besides IFN-γ, we suggest that high IL-2 production in ASY could be related to infection resolution

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EXPERIMENTAL INFECTION OF *SUS SCROFA* (DOMESTIC PIG) WITH *TRYPANOSOMA CRUZI*

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Trypanosoma cruzi is responsible for producing Chagas diseases in humans.Its pathophysiology is not yet well understood.Therefore, our goal was to assess the domestic pig as an experimental model to study Chagas disease resulting from infection with the Bolivia strain. Five 60day old mixed breed female pigs were randomly inoculated, four with the Bolivia strain and one with saline.Two animals were infected with $1x10^6$ trypomastigotes/kg b.w and $5x10^6$ trypomastigotes/kg.b.w administered intravenously, and two pigs received the same doses by the intradermal route.Microconcetration method was used to evaluate parasitaemia from 7 days post-inoculation(dpi). IgM and IgG were assessed by EAE-ELISA from serum samples. Necropsy was performed at 150 dpi and tissues were collected to determine tissue damage and presence of amastigotes by histology. Clot samples and tissues were analyzed by PCR (kinetoplast DNA of *T. cruzi*). All infected animals showed a parasite burden from 10 dpi.This decreased in three groups at 35 dpi; animals inoculated with $1x10^6$ trypomastigotes/kg body weight by the intravenous route showed a reduction in parasitaemia at 45 dpi.All infected animals were parasitaemia-negative from 50 dpi. IgM was detected from 5 dpi in 3/4, 4/4 were positive at 10 dpi. Peak in IgM occurred between 20 and 25dpi and was maintained until 55 dpi, being negative at 90 dpi. IgG increased from 20dpi until 75 dpi and remained elevated at the end of the experimental period. PCR detected positivity in blood from infected animals from 3 to 60 dpi; however, at 75 dpi only 2 animals were positive.One animal remained positive up to 150 dpi.Tissue PCR found 3/4 infected animals were positive cerebrally, 2 were spleen positive, and only 1 was positive in the small intestine. All were negative in the heart, kidney, and large intestine. Nests of amastigotes were not found in the tissues. Slight lymphocytic perivasculitis was observed in the pericardial and meninx area. Furthermore, the splenic capsule showed increased lymphoid nests and mild disintegration of the white and red pulp. A slight focal glomerulonephritis with perivasculitis,mainly lymphocytic, were observed in the renal tissue. The control animal was negative in all analyses performed. Our study concluded that the pig can be used as an experimental model for Chagas disease, and suggests that with increasing time post-infection, the pig can develop similar chronic pathologies to humans.

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MULTIPLEX REAL-TIME PCR FOR DETECTION OF SPECIES OF SUBGENUS VIANNIA AND *LEISHMANIA* (L.) *DONOVANI*

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Leishmaniasis can be caused by over 20 distinct species of the genus *Leishmania.* The diseases present a wide spectrum of symptoms which

may be confused with other etiological agents. Species identification is needed, especially in geographic regions endemic for both cutaneous and visceral forms of the disease. In this study we describe three real-time multiplex PCR assays for detection of several species of *Leishmania* of the subgenus *Viannia* and *L. donovani* complex. The 313 samples used in this study were divided in two groups. Group I: 220 DNA samples from blood and tissue from dogs and humans positive for several species of *Leishmania*. Group II: 93 DNA samples from blood, tissues and stools from humans that were negative for *Leishmania*, but positive for others parasites; i.e., *Plasmodium* spp., *Acanthamoeba spp., Balamuthia mandrillaris, Naegleria fowleri*, *Entamoeba histolytica, E. dispar, and Trypanosoma cruzi.* A generic probe targeting 18SrRNA labeled with the CY-5 was used to identify the *Leishmania* spp. at genus level. Specific probes targeting the *Leishmania* actin gene labeled with FAM and HEX were used to discriminate subgenus *L. Viannia* and *L.* (*L.*) *donovani* complex respectively. The reactions were performed on an ABI 7500 Real Time PCR System (Applied Biosystems). The CY-5 generic reaction was able to detect 59 out of 66 (89.4%) and 100 out of 110 (90.9%) isolates previously identified as positive for the subgenus *Viannia* and *Leishmania (L.) donovani*, respectively. Using the FAM reaction 60 out of 66 (90.9) of the subgenus *Viannia* isolates were detected and by the HEX reaction 104 out of 110 (94.5%) of isolates of *Leishmania* (*L.*) *donovani* complex were detected. No cross reaction among the specimens from subgenus Viannia and *L.* (*L.*) *donovani* complex were observed. Isolates of *L. (L.) amazonensis, L. (L.) mexicana* and *L. (L.) major* were positive only for the CY-5 probe. The other species belonging to the subgenus *Leishmania*, such as *L. (L.) tropica* and *L. (L.) aethiopica*, also tested positive for both marker CY-5 and HEX. The 93 samples negative for *Leishmania spp*. showed no Ct in all analyses performed. The multiplex PCR using the FAM and the HEX probes was effective in discriminating species of the subgenus *Viannia* and the *L* (*L.*) *donovani* complex. The results indicate that the method could be useful for simultaneous detection of *Leishmania* species from subgenus *Viannia* and *L. donovani* complex.

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EVALUATION OF STABILITY OF THE PHENOTYPIC AND GENOTYPIC CHARACTERISTICS OF WHO REFERENCE STRAINS AND CLINICAL ISOLATES OF *LEISHMANIA BRAZILIENSIS* AND *L. PANAMENSIS*

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Methodologies used in the study of *in vitro* drug susceptibility, diagnostic tests and identification of *Leishmania* species require the use of control reference strains that do not lose their phenotypic and genotypic characteristics after successive passages *in vitro*. The number of passages of the *in vitro* strains can identify *Leishmania* phenotypes with increased *in vitro* growth potential. This could affect the precision to establish the logarithmic growth phase and the phenotypic and genotypic characteristics of the parasite. The objective of this study was to evaluate the behavior of the growth curve, the isoenzyme and monoclonal antibodies profiles, the amplification and RFLP of the mini-exon and Hsp70 genes in WHO *L/ braziliensis* and *L. panamensis* reference strains as well as in 2 clinical isolates in different culture passages (1, 5 and 10). We found that behavior of growth in the logarithmic phase and the percentage of viable parasites in the reference species and in the clinical isolates was similar in passes 5 and 10. However the behavior in passage 1 was different, which can be explained because the parasite population is not in the same phase of growth at the beginning of the culture. The genotypic characterization through PCR-RFLP using molecular markers for the mini-exón gene and the HSP70 genes showed no differences between the subcultures studied. In conclusion, no differences were found in the phenotypic and genotypic characteristics evaluated in the reference strains and clinical isolates in passages 5 and 10 of culture. Therefore, given the phenotypic and genotypic stability, it is recommended to use cultures up to passage 10 *in vitro* studies.

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CALMODULIN INTERGENIC SPACER: A USEFUL MARKER FOR THE IDENTIFICATION AND CHARACTERIZATION OF *LEISHMANIA* SPECIES

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Calmodulin gene modulates the calcium metabolism in various cellular activities on tripanosomatides. Although this gen is evolutionary highly conserved in tripanosomatides, it has been demonstrated that the 3' UTR regions in *Trypanosoma cruzi,* present genetic variations capable to regulate the expression. Furthermore, mutations of calmodulin intergenic spacer have been demonstrated to be specific for *T. cruzi* major groups. In this study we demonstrate that the segment of calmodulin gene containing both Untranslated Regions (3' and 5'UTR), may be used as molecular marker to distinguish between *Leishmania* species, after a sequence analysis. *Leishmania* reference strains and clinical isolates from Panamanian patients were evaluated. A set of primers were designed based on the open reading frame of the calmodulin gene to amplify the 3' UTR, the intergenic region and the 5' UTR. As expected, *Leishmania Viannia* and *L. mexicana* reference strains appear to have similar calmodulin gene organization, which according to *L. braziliensis* genome sequence comprises three copies of the calmodulin orf and two intergenic spacer: 1.2 kb and 1.6 kb bases. Only two copies of this gen appear to be present in *L. chagasi* genome. PCR conditions were set up to favor the amplification of the smaller fragment of the spacer (~ 1230 bp). Sequence analysis of cloned PCR products revealed that this region presents genetic variations that clearly identified each of the *Leishmania* reference species evaluated. Parasites isolated from clinical samples were classified as *L. Viannia panamensi*s based on the mutations pattern observed. Our preliminary results indicate that the intergenic spacer of the calmodulin gene is a useful molecular marker for the identification and characterization of *Leishmania* species.

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A FAMILY OF SEVEN CYTOCHROME B5 REDUCTASES AS NOVEL THERAPEUTIC TARGET IN *LEISHMANIA MEXICANA*

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Leishmania parasites are opportunistic protozoan flagellates that cause devastating and often fatal diseases such as mucocutaneous or visceral leishmaniasis in much of the tropical and subtropical world. Emerging drug resistance is one of the problems in leishmaniasis treatment, contributed by enzymes involved in the detoxification of pharmacological agents and other xenobiotics. One such enzyme, cytochrome b5 reductase (Cb5r), has a high pharmacological significance owing to its role in fatty acid elongation, ergosterol (*Leishmania*) or cholesterol (human) biosynthesis, and cytochrome P450-mediated detoxification of xenobiotics. We have identified a new family of seven Cb5r isoforms in *L. mexicana*, in contrast to the one major isoform in humans and only two isoforms in fungi (CBR1, MCR1). Phylogenetic analysis revealed that one *L.mexicana* isoform, LmexCb5r-7, has closest homology to human Cb5r and fungal CBR1, while the other six isoforms form three separate independent clades. LmexCb5r-1 and 2 are most distant from human Cb5r and are located in tandem repeat on the same chromosome 22, while the other five isoforms are each located on separate chromosomes. We have cloned LmexCb5r-1 and 2 and expressed as recombinant His_6-tagged protein in *E.coli* for subsequent biochemical and pharmacological analysis. Furthermore we modeled the molecular structure of all seven LmexCb5r isoforms *in silico* based on the crystal structure of the mammalian Cb5r enzyme from human and rat for rational drug design. Biochemical analysis of recombinant LmexCb5r-1 and 2 revealed similar substrate affinities

for NADH (K_m = 21.3±1.9 μM and 23.5±5.1 μM, respectively) but 7-fold higher V_{max} value in LmexCb5r-1 compared to LmexCb5r-2. We are currently also characterizing the LmexCb5r-7 isoform closest to human Cb5r. Interestingly, human Cb5r has about 4-fold higher substrate affinity (K_m = 6μM) and approximately 500-fold higher V_{max} value compared to LmexCb5r-1, and it hence will be particularly interesting to characterize LmexCb5r-7 closest to the human enzyme.

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HIGH LEVELS OF FETAL ANEMIA AMONG NEWBORNS IN RURAL NORTHEASTERN GHANA ARE INDEPENDENTLY ASSOCIATED WITH MULTIGRAVID MOTHERS AND *PLASMODIUM FALCIPARUM* INFECTIONS IN CORD BLOOD

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Malaria during pregnancy entails a risk of severe anemia in the mother, and low birth weight in her baby, but the relationship between placental malaria and fetal anemia is unclear. We hypothesized that fetal anemia (FA, Hb <12.5 g/dL), seen in 22% of live births during 2007 in rural northeastern Ghana, was directly related to placental malaria, and could be reduced by Fansidar-based Intermittent Preventive Treatment during pregnancy (SP-IPTp). This was investigated retrospectively in a cohort of 2096 newborns using polymerase chain reaction to detect *P. falciparum* in their cord blood samples. Results were analyzed against characteristics of the mother and baby using multivariate logistic regression. We detected *P. falciparum* in 18% of cord bloods, mainly among first time mothers (OR = 1.57, 95% CI: 1.18, 2.08), low birth weight (LBW, <2500g) infants (OR = 2.16, 95% CI: 1.65, 2.84), and in babies born during wet season (OR = 3.071, 95% CI: 2.321, 3.921). Unexpectedly, FA was independently associated with deliveries by multigravidae (OR = 1.85, 95% CI: 1.30, 2.62), and associated with cord blood *P. falciparum* infections in their newborns (OR = 1.83, 95% CI: 1.27, 2.64). There was no significant effect of SP-IPTp seen on reduced levels of either LBW or FA, but the risk of cord blood malaria infection was significantly higher for infants of mothers who used no SP-IPTp (OR = 1.61, 95% CI: 1.13, 2.29), and drug effect was dose-dependent: Optimal SP-IPTp, based on three Fansidar doses, was significantly more protective than suboptimal IPTp (1-2 doses) against malaria infection (OR = 0.64, 95% CI: 0.49, 0.84). Fetal anemia in northeastern Ghana occurs at a disproportionately high rate in babies born to multigravid mothers and is exacerbated by malaria during late pregnancy when fetal oxygen demand is greatest. The importance of IPTp for pregnant multigravidae women, and its benefit for their babies has been questioned, and debated, but seems clear and well-justified in light of these results.

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PUTATIVE PARASITE-ENCODED ADHESIN EXPRESSED ON THE SURFACE OF *PLASMODIUM YOELII* 17X INFECTED RETICULOCYTES

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Adherence of *Plasmodium falciparum* infected erythrocytes to vascular endothelial cells contributes to pathology and disease severity. This adherence is primarily mediated by var gene-encoded *P. falciparum* erythrocyte membrane protein-1 (PfEMP-1), which is unique to *P. falciparum*. Red blood cells (RBCs) infected with other malarial parasites, including *Plasmodium vivax*, are also reported to adhere to vascular endothelium, although the parasite-encoded RBC surface proteins mediating this adherence are less well studied. Using the reticulocyte-restricted parasite *P. yoelii* 17X, we developed an adherence assay that allows for isolation of parasites from adherent and non-adherent reticulocytes. Using *P. yoelii* DNA microarrays, we identified six genes encoding putative adhesins whose expression was consistently up-regulated in parasites from adherent versus non-adherent reticulocytes. Members of the yir and pyst-a multigene families, which are predicted to encode RBC surface proteins in *P. yoelii*, were not included among these putative adhesins. We focused on PY04120, which encodes a ~193 kDa protein that is highly conserved across malarial species. We have expressed a 26 kDa fragment of the PY04120 protein in *E. coli* and have generated rabbit antisera. We can detect PY04120 protein in a *P. yoelii* -infected reticulocyte membrane protein (PyRMP) preparation and immunofluorescence using live, unfixed *P. yoelii* 17X infected RBCs (iRBCs) indicates that PY04120 is expressed on the surface of a subset of schizont-stage infected reticulocytes. Sera raised against PY04120 can also partially block adherence of P. yoelii 17X iRBCs to the mouse endothelial cell line bEnd.3. These results indicate that the interaction of PY04120 with endothelial receptors may contribute to adherence of iRBCs in host tissues. Studies to generate a PY04120 gene knock out in P. yoelii 17X to further characterize this putative adhesin are in progress.

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IMPACT OF HOST SERUM IRON ON THE ERYTHROCYTIC STAGE OF THE MALARIA PARASITE

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Iron is an essential nutrient for *Plasmodium falciparum*. The malaria parasite requires iron for DNA synthesis, glycolysis, pyrimidine synthesis, heme synthesis and electron transport. The host RBC contains 100fg (20mM) of iron, however, the majority of it is sequestered in heme which is incorporated into hemoglobin. Rather than releasing the iron from the host heme, the parasite synthesizes hemozoin, an inert crystal of heme molecules. There is no evidence, that the parasite has the heme oxygenase activity necessary to release iron from heme and there have been conflicting reports about whether or not the parasite is able to utilize human transferrin. No plasmodial iron storage proteins, siderophores or chelators have been identified. Thus, despite an obvious iron requirement, it remains unclear what iron source *P. falciparum* is able to acquire and utilize during the erythrocytic stage of infection. In the present study we report that the availability of extra-cellular iron in the form of Fe citrate and human transferrin impacts the growth of erythrocytic stage *P. falciparum*. We show that the bioavailable iron and reactive oxygen content of parasitized erythrocytes is dynamic and subject to the availability of extracellular iron. Additionally, using confocal microscopy, we study the binding of fluorescently labeled human transferrin to late stage *P. falciparum* trophozoites and schizonts.

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MRI OF THE BRAIN IN ADULT PATIENTS WITH CEREBRAL AND SEVERE MALARIA

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Magnetic Resonance Imaging (MRI) allows detailed study of the pathogenesis of cerebral malaria in living patients but its availability in malaria-endemic areas is limited. A high powered scanner allows

assessment of cerebral blood flow, blood volume, oedema, ischaemia, haemorrhages and blood brain barrier integrity; and by magnetic resonance spectroscopy (MRS) metabolite levels and neuronal injury. A recent study in African children using a 0.35 tesla scanner found gross abnormalities to be common, especially brain swelling and ischaemia. There is accumulating evidence that the pathogenesis of cerebral malaria differs between adults and children. In adults, there have been several case series of MRI in patients with cerebral malaria but no systematic study. We performed a descriptive study of the morphological and functional changes of the brain using high-powered MRI and MRS in patients with severe and cerebral malaria. Patients were enrolled from 2009-2011, in Chittagong Medical College Hospital, Chittagong, Bangladesh. Sequences included T1, T2, Flair, DWI, GRE and magnetic resonance spectroscopy (MRS) including brain lactate levels. Scanning was done as early as possible within maximum 48 hours of admission. All patients also had a full clinical assessment including blood tests for haematology, biochemistry, acid-base status, lactate parasitaemia and HRP2. Retinal photography was performed to assess malaria retinopathy. 43 adult patients with severe malaria (30 with cerebral and 13 with noncerebral severe malaria) were enrolled, 26 were scanned in a 1.5 tesla and 17 in a 0.3 tesla scanner. 12/43 (28%) were fatal and 20/43 (47%) had hyperlactaemia. 80% of patients had abnormalities on 1.5 tesla MRI. These were mostly mild and included subtle cerebral oedema, ischaemia, mildly raised brain lactate and choline levels and venous congestion. Abnormalities were commoner and more marked in patients with cerebral malaria but less prominent than those found in Malawian children. The cerebral oedema found was thought not severe enough to cause coma. Ischaemia on DWI was mostly restricted to those with coma. This suggests cerebral oedema is not an important contributor to coma in adult cerebral malaria and the pathogenesis may be different from in children. Obstruction of the cerebral microcirculation by sequestered parasites is likely to be the cause of the brain ischaemia.

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BIOLOGICAL DISORDERS IN SUB SAHARAN CHILDREN (SENEGAL) WITH UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA TREATED BY ACTS

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Biological disorders are frequent in children acute uncomplicated *Plasmodium falciparum* malaria but not well studied. Several abnormalities can be present before the infection. Others as started at the beginning of the disease. These disorders can be worsened by drugs used for the treatment (drug toxicity). The objective of this study was to analyze biological parameters (hematologic and biochemistric) for children with acute uncomplicated *P. falciparum* malaria at the enrollments and after antimalarial treatment. Data on age, hematologic parameters (hemoglobin levels or hematocrit, platelet counts), biochemistry parameters (Alanine amino transferase, Aspartate amino transferase, bilirubine and creatinin) and treatment were collected and analysed. Univariate and multivariate analysis were done at day 0 and day 7 after treatment in overall patients and taking in account ACT used for treatment (ASAQ, AL, ASMQ, DHA-PQ). Data for 720 patients under 10 years were analyzed. Mean age of the patients was 9.4 years. Anemia was found in 72.78% (524/720) at D0 versus 80.14% (577/720) at D7 (p=0.001). Thrombocytopenia was present in 386 patients (53.61%) at D0 versus 47 patients (6.53%) at D7 (p=0.03). Regarding ALAT and ASAT, it appears that levels were higher at D0 compared to D7 (p=0.001). Same results were obtained in comparing bilirubine (72.78% at D0 vs 397 55.14% at D7) and creatinine (p=0.001). Positive impact of ACT's was not found in improving anemia between D0 and D7: 68.18% versus 79.26% for AL (n=352 p=0.0001); 68.3% versus 60% for DHA-PQ (n= 120, p=0.0001); 78.12% versus 88.12% for ASMQ (n=160, p=0.001) and 87.50% versus 96.59% for ASMQ (n= 88, p=0.004). Decrease and a return to normal of liver enzymes and creatinine were more significant in DHA-PQ group following by ASMQ and AL. In conclusion, our results showed that hematological disorders are common at the beginning of acute uncomplicated *P. falciparum* malaria in children and they persist up to 7 days after treatment with different ACT's. However, these drugs didn't affect biochemistry parameters.

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TRANSCRIPTIONAL PROFILING VALIDATES MECHANISMS AND BIOMARKERS OF ERYTHROPOIETIC SUPPRESSION IN SEVERE MALARIAL ANEMIA

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Malaria is a major global cause of anemia. In malarial anaemia, dyserythropoiesis and erythropoietic suppression can prevent adequate compensation for erythrocyte destruction, but these processes are poorly understood. To study their mechanistic basis, we collected 286 bone marrow (BM) aspirates in the context of a large case-control study of anemia in Mozambique and examined a wide range of clinical, hematological, biochemical, immunological, microbiological and genetic markers. We developed novel flow-cytometric analysis and a transcriptional profile based on erythroblast-specific gene expression to quantifiy erythropoiesis. Both measures correlate well with classic morphological quantification in stained bone marrow smears (both $p<0.0001$). Whereas peripheral reticulocyte numbers showed no correlation with bone marrow erythropoiesis in this cohort, the red cell distribution width (RDW), a measure of variance in erythrocyte volume, was validated as a novel peripheral biomarker for this important response ($p<0.0001$). Using these measures of bone marrow erythropoietic differentiation, samples were categorized as having low or high erythropoiesis relative to hemoglobin levels. A first pass analysis revealed that both bacteremia and HIV infection were more frequent in samples with low erythropoiesis. Unexpectedly, infection with malaria parasites was equally common in samples with low and high erythropoiesis with high erythropoiesis linked to severe anaemia in the presence of hemolysis. These findings are currently being validated and extended using rigorous uni- and multivariate statistical testing. Erythropoietin (EPO) insufficiency has been debated as a cause of erythropoietic suppression. Our data showed a strong fit of EPO concentrations with Hb levels but not with the level of erythropoiesis ($r^2=0.65$ vs. 0.29). Thus, these data provide superior direct and peripheral biomarkers of erythropoiesis, differentiate several groups of erythopoietically suppressed bone marrow and provide a rich data set for the analysis of transcriptional footprints indicative of underlying mechanisms. Such findings may inform future therapies for this serious disease.

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COMMON RED BLOOD CELL POLYMORPHISMS AND *IN VITRO* GROWTH OF *PLASMODIUM FALCIPARUM*

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It has been almost 60 years since it was proposed that the high frequencies of hemoglobinopathies seen in Africa and Asia were the result of selective pressure in response to *Plasmodium* species (The malarial hypothesis). Abundant evidence exists to support hemoglobin S (HbS) and to a lesser extent for hemoglobin C (HbC) and hemoglobin E (HbE) as examples of hemoglobinopathies conferring a reduced risk of severe malaria depending on an individuals' heterozygous state. The exact mechanism for this protection remains unclear. Studies have suggested

that the invading parasites cannot survive within variant red blood cells due to a number of different possible mechanisms. However, variations in in-vitro cultivation procedures make comparisons between studies problematic. We believe that oxidative stress may play a role in parasite survival in hemoglobin variant red blood cells. We describe and compare the *in vitro* growth of *P. falciparum* in red blood cells from individuals with defined hemoglobin (Hb) β chain variants, HbA, HbC, HbD/G, HbE, HbF, HbS, in a controlled oxygen atmosphere. Flow cytometry was used to enumerate infected cells after 72 hours of culture. Under low oxygen concentration (2%), we found a marked decrease in parasite growth in both the HbAS and the HbF RBC cultures as compared with HbAA. While growth in HbAC RBCs was slightly inhibited and no consistent pattern in parasite growth was seen in cultures using the HbD/G variant. Our results using HbAE red blood cells were inconclusive. Future experiments will be done under various oxygen concentrations. Our studies will help to better understand the complex interaction between malaria parasites and human red blood cells.

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SINGLE CELL GENOMICS FOR MALARIA PARASITES

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Malaria parasite infections are composed of millions of individual parasite cells. Infections frequently comprise multiple genotypes or species, and many parasite species are not possible to culture in the laboratory. As a consequence, next generation sequence data from malaria infections often comprises a soup of different sequences and accurate inference of component parasite haplotypes is problematic. To dissect the genetic composition of these complex mixtures, methods capable of genotyping (and phenotyping) single parasites would be ideal. Using approaches adapted from the cancer and bacterial literature, we have developed single cell methods – isolating single cells using fluorescence activated cell sorting (FACs), followed by whole genome amplification and array based genotyping or Illumina sequencing - to generate genomic data from single infected red blood cells. To demonstrate the utility of these methods we made mixtures of *Plasmodium falciparum* 3D7 and HB3 laboratory parasites lines, and then isolated and genotyped single cells from these mixtures. We are now further refining these methods to examine the genetic composition of natural infections containing *P. falciparum* and *P. vivax*.

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DEVELOPMENT OF A *PLASMODIUM CHABAUDI* RODENT MODEL SYSTEM FOR PLACENTAL MALARIA

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Pregnancy associated malaria (PAM) is associated with placenta pathology and poor pregnancy outcome but its mechanism is poorly understood. PAM has been studied in rodents for a long time now, but we still do not have a good existing rodent or non-human primate model. Here we propose a rodent model system for *Plasmodium chabaudi* infection during pregnancy in C57BL/6 mice and in African thicket rats; the latter are the natural rodent hosts for malaria. In the process we have also tried to study the pathogenesis and immune responses during placental malaria in these animals. We looked at the pathogenesis of the malaria infection before and during the pregnancy using two different strains of *P. chabaudi*, the AS and the CB. The mice when infected with the AS strain did not show any parasite recrudescence during the pregnancy but the mice when infected with the CB strain of *P. chabaudi* seems to develop chronic infection and did show parasite recrudescence during the pregnancy. We also got some interesting results when we transferred our understanding from these mice into the thicket rats. The development of these models and our findings from them will open new insights into the understanding of malaria during pregnancy.

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DEPLETION OF CD8 T CELLS DELAYS CLEARANCE OF PARASITEMIA AND INCREASES ANEMIA IN A RAT MODEL OF MALARIAL ANEMIA

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MSP7 is a malaria parasite gene family used by blood stage merozoites to invade erythrocytes. It is conserved through the genus *Plasmodium*. We have previously shown that deletion of a single *msp7* gene, reduced anemia despite a small change (3 to 2%) in peak, peripheral parasitemia in rats infected with *Plasmodium berghei,* as reported previously. We now report that in the spleen, the peak parasite load is 4-fold higher for wild-type parasites relative to Δ*mps7* counterparts. This occurs at day 8 post infection. At d10, which is also the onset of anemia, the spleen parasite load was reduced by 40-fold for wild-type parasite infected rats, compared to a ten-fold drop for Δ*msp7* mutant-infected animals. Analysis of gene expression, suggested that at day 10, there is elevation of granzymes in the spleen of rats infected by wild-type parasites compared to Δ*msp7* mutants. Cellular analysis indicated no difference in NK cells but a two-fold elevation in number of CD8 cytotoxic T cells. Immunohistochemistry revealed that by day 10, the CD8 T cells accumulated principally in the red pulp zone. Depletion of CD8 T cells (but not CD4 T cells), had no effect on the rise of parasitemia, but delayed parasite clearance from the periphery, increased parasite load in the spleen, and exacerbated anemia. These data suggest that CD8 T cells control parasite clearance. Further, persistence of parasitemia increased anemia. Current studies are focused on the mechanism by which CD8 T cells eliminate infected erythrocytes and how MSP7 engages CD8 T cell dynamics in the spleen.

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SUSCEPTIBILITY OF OLD VS. YOUNG ERYTHROCYTES TO *PLASMODIUM FALCIPARUM*

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Plasmodium falciparum is known to cause severe disease in humans and accounts for one million deaths per year. The virulence of this parasite has been in part attributed to its ability to promiscuously invade young and old erythrocytes. However, it has been observed that young red cells are more susceptible to invasion. The parasite is known to utilize the sialic acid on glycophorins as a main invasion pathway and a sialic acid-independent invasion pathway utilizing complement receptor 1(CR1) was recently identified. As erythrocytes age they lose glycophorins and CR1 which could account for their lower susceptibility to invasion. We hypothesized that if aged red cells are less susceptible to invasion by *P. falciparum*, this may be due to decreased availability of these receptors. To test our hypothesis erythrocytes were separated based on age using a discontinuous Percoll gradient. Differential susceptibility to invasion of each red cell population was tested *in vitro* using invasion assays with the

P. falciparum 7G8 strain. We observed no difference in the susceptibility to invasion among red cells of different ages and no shift in the use of receptors with age.

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SUSTAINING THE GAINS AND PROGRESSING TOWARDS MALARIA ELIMINATION IN KWAZULU-NATAL PROVINCE, SOUTH AFRICA

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Malaria transmission increased nearly 10-fold from 1995 to 2000 in KwaZulu-Natal Province, South Africa, due mainly to vector resistance to pyrethroids and parasite resistance to sulfadoxine-pyrimethamine. Transmission subsequently dropped by 77% between 2000 and 2001 and by 99% between 2000 and 2011 (from 41,786 cases to 598). This marked decline was due to a combination of interventions, including the reintroduction of DDT in 2000, introduction of ACTs in 2001, cross-border collaboration with Mozambique and Swaziland through the Lubombo Spatial Development Initiative (LSDI), and intensified surveillance. However, cases in KwaZulu-Natal have plateaued between 2007 and 2011 with an average of 509 cases reported each year. The lowest transmission was experienced in 2010 with 380 reported cases followed by an increase to 598 cases in 2011, of which 51% were classified as imported - 69% of which were from Mozambique, 20% were local, and 29% were unclassified. This upsurge is concerning and may be due in part to the cessation of the LSDI. To sustain the gains, collaboration with Mozambique must continue to reduce imported cases, coverage of interventions in the province must remain high, and insecticide and drug susceptibility must be monitored to avoid resistance. As part of South Africa's move toward elimination by 2018, KwaZulu-Natal must not only sustain efforts but also transform its malaria programme to eliminate malaria systematically from its transmission foci. With the lowest local transmission in South Africa (121 local cases in 2011), KwaZulu-Natal can be the first province to eliminate malaria through prompt and robust collection and use of information, focal IRS, proactive screening and treatment among high risk groups, and effective response to every malaria case in receptive areas. Maintaining a strong vertical programme especially for IRS to enable rapid and extensive intervention will be critical while working toward integration of surveillance, IEC, and case management into the broader health system to prevent reintroduction following elimination.

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DEVELOPING THE MALARIA ELIMINATION TOOLKIT: A MONITORING AND EVALUATION TOOL FOR CASE INVESTIGATION AND REACTIVE CASE DETECTION

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Active case detection (ACD) strategies are recommended by the World Health Organization and are implemented in many malaria endemic countries worldwide as a critical part of malaria elimination programs. Strategies include determining the origin of infection, case investigation, and responding to a locally acquired case of malaria, known as reactive case detection (RCD). RCD strategies differ across countries, ranging from screening and treating household members to whole communities of the identified index case, and may include forms of vector control. Due to the programmatic and financial considerations of implementing RCD, malaria control programs need to be able to measure the operational effectiveness and cost-efficiency of their RCD strategies, as well as compare their RCD strategies to those of other countries. We have thus developed a standardized tool to monitor and evaluate the technical and operational effectiveness and cost-efficiency of RCD in malaria endemic settings. With our evaluation tool, RCD effectiveness and efficiencies are measured with process and outcome indicators, such as time lapsed between case diagnosis and investigation; percentage of cases investigated; proportion of additional cases identified through RCD; and screening and treatment. The evaluation tool gathers qualitative survey data, which are collected from focus group discussions with surveillance personnel concerning information flow and implementation of strategies. Financial analysis of surveillance-related expenditures are analyzed to determine the primary cost drivers and operating costs for RCD, resulting in an estimate for total cost per investigation and the cost per additional case of malaria actively detected. Findings from the evaluation tool can inform and test new programmatic interventions within endemic countries to mitigate challenges and strengthen the RCD surveillance program. The evaluation tool will be piloted in low transmission countries in the Asia Pacific and southern Africa with the goal to expand its implementation on a larger scale in the future.

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ACTIVE CASE DETECTION TOWARDS MALARIA ELIMINATION: OPERATIONAL FINDINGS FROM MPUMALANGA PROVINCE, SOUTH AFRICA

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To achieve malaria elimination by 2018, South Africa's malaria control programme is prioritizing intensified surveillance efforts with strong emphasis on Active Case Detection (ACD). Mpumalanga is one of three endemic provinces in South Africa and has a functional ACD system. The National and Mpumalanga Malaria Programmes jointly evaluated the provincial ACD system to identify best practices and inform ACD efforts in South Africa. The evaluation was conducted in the highest burdened municipalities, Bushbuckridge and Nkomazi, and consisted of qualitative and quantitative data collection methods and analysis. Questionnaires were administered to all direct and peripheral ACD staff in early 2012, and quantitative data was extracted from the provincial Integrated Malaria Information System (IMIS) and measured against defined indicators. Case investigation teams, consisting of one case investigator and at least two assistants, are responsible for conducting ACD, including case investigation. From July to December 2011, each team in Bushbuckridge was responsible for a mean of 29 malaria cases, with a range from 21 to 41 cases per team, while each team in Nkomazi was responsible for a mean of 71 cases with a range of 22 to 145 cases per team. The median number of days from diagnosis to ACD in Bushbuckridge was 4 days and 6 days in Nkomazi. The median number of days from diagnosis to entry of the ACD forms into the IMIS was 14 days and 6 days in Bushbuckridge and Nkomazi, respectively. This latter discrepancy is most likely due to the data capture centre being based in Nkomazi. Best practices include ACD-dedicated supervisors and case investigation teams and the placement of assistants at high burden health facilities in Nkomazi to complete notification forms and facilitate ACD. To improve operational efficiency, the malaria programme should consider implementing mapping techniques, improving communication between Bushbuckridge and the data capture centre, and restructuring teams to optimize human resource capacity toward ensuring a robust ACD system for elimination.

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ASSESSING THE EFFECTIVENESS OF ARTEMISININ-BASED COMBINATION THERAPIES IN RURAL GHANA

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The WHO recommends Artemisinin-based Combination Therapies (ACTs) for the treatment of uncomplicated *falciparum* malaria. Post licensure studies to monitor effectiveness of new drugs when delivered in real life settings are often lacking in Africa. We assessed the effectiveness of ACT policy five years following its implementation in the middle belt of Ghana. Both quantitative and qualitative surveys were carried out to assess uptake of ACT within the Kintampo Health and Demographic Surveillance System between October 2009 and July 2011. Participants were residents who reported of fever/malaria with a two-week recall. Finger prick blood sample for malaria microscopy were also collected at randomly selected households and health facilities during patients exit interviews. The population prevalence of malaria parasitemia was 28.2% (95% CI: 25.7-30.4) and 37.1% (95% CI: 30.3-44.3) among children less than five years old. About 40.6% (95% CI: 38.1-44.0) of patients who reported fever sought health care within 24 hours. Only 46.9% (95% CI: 40.5-53.4) of fever cases were investigated for malaria. About 62.1% (95% CI: 55.6-68.4) of patients were over-treated with ACTs based on reference blood slide results. About 48.0% (95% CI: 42.9-53.2) of patients prescribed ACTs failed to comply with medication instructions. Negative perceptions associated with the use of ACTs were also reported by some communities. Limiting factors of the health systems and negative community perceptions are potential impediments to uptake and adherence to ACTs. These do not only erode the benefits of efficacious ACTs but has potential for development of resistant strain of malaria parasites.

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HIGH-THROUGHPUT ASSESSMENT OF THE INFECTIOUSNESS OF NATURALLY INFECTED GAMETOCYTE CARRIERS TO *ANOPHELES* MOSQUITOES

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The most important biological outcome measure in future vaccine or drug-based transmission reducing trials in endemic malaria areas is the proportion of infected mosquitoes in direct (membrane) feeding assays. These assays were previously found to give highly variable results and this is an issue that needs to be solved if it is going to be used in the evaluation of the efficacy of future GCP clinical trials. Here, we study the reliability of two different methods in determining the proportion of infected mosquitoes post membrane feed. Fifty gametocyte carriers of 2-12 years of age were asked to donate each a 5mL blood sample to be distributed over four minifeeders for experimental mosquito's infection. One hundred mosquitoes were allowed to feed on each feeder for exactly 15 minutes. On Day 7, fifty mosquitoes from each experiment were dissected for microscopic detection of oocyst in infected mosquitoes. The sensitivity of an ELISA based-detection method of circumsporozoite protein in oocysts was determined for mosquitoes on day 7, 10 or 14 after feeding. Overall and as detected by microscopic examination, 66% (33/50) of children infected at least one mosquito. In total, 14.12% (1,059/7,496) of the mosquitoes were infected with a mean of 7.76 (range 1-110) oocysts per midgut. ELISA-based detection of oocysts on day 10 and 14 was more sensitive than on day 7 post-feeding and was strongly correlated with microscopic examination of midguts. In conclusion, these findings suggest that early microscopic examination of midguts for determining the biological outcome measure in transmission reducing interventions may be less informative. ELISA-based detection of oocyst, a high-throughput method as compared to microscopy appears to be more sensitive and convenient for the evaluation of transmission control interventions in the context of malaria eradication.

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MODELING THE IMPACT OF FIRST- AND SECOND-GENERATION MALARIA VACCINE ROLL-OUT STRATEGIES

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Given the desire to develop new tools to pair with existing drugs and vector control for the purpose of eradicating malaria from the most endemic regions of the globe, the advent of new malaria vaccine candidates is encouraging. Even with present prospects of only partially protective immunity, pre-erythrocytic and gametocyte-blocking vaccines may be useful as components of multi-intervention eradication strategies in certain circumstances. We present a systematic study of the utility of potential malaria vaccines within the EMOD model, a comprehensive model of the vector life cycle coupled to a detailed mechanistic representation of intra-host parasite and immune dynamics. Over a range of vaccine efficacies and roll-out scenarios, the impact of introducing malaria vaccines, alone and in conjunction with large-scale ITN coverage, is demonstrated for a variety of transmission intensities and entomological behaviors and ecologies. We present reductions in incidence among the protected population as well as community-level reductions in transmission. Our results demonstrate the types of settings where first-generation malaria vaccines are likely to provide the greatest impact, and provide estimates of efficacy targets for second-generation vaccines as a function of desired impact.

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BALANCING INTERVENTIONS WITHIN FIXED-BUDGET MALARIA CONTROL PROGRAMS IN AFRICA

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Malaria control programs often work within a fixed budget. In order to maximize economic and social benefit, they must balance their efforts among a variety of tools (indoor residual spraying, insecticide-treated nets, and antimalarial drugs). We study how this balance changes across different levels of endemic transmission, vector behavior, intervention coverage, and regional income level. We use the stochastic agent-based EMOD model to quantify the impact of a program on malaria transmission, mortality and morbidity, and evaluate how costs may scale as a function of coverage for each type of intervention considered.

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STEPS TOWARDS MALARIA ELIMINATION: INTEGRATING POPULATION-WIDE TEST AND TREAT CAMPAIGNS INTO MALARIA CONTROL IN ZAMBIA

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The current Zambia National Malaria Strategic Plan (NMSP) 2011-2015 targets elimination of malaria in 5 areas/districts by 2015. Based on data from Malaria Indicator Surveys (MIS's) carried out in 2006,2008, and 2010, the NMCP stratified the country into 3 epidemiological zones. The National Malaria Control Program (NMCP) and partners used a DHIS 2 mobile phone system to increase information on malaria prevalence in specific district health facility catchment areas and the information generated from this system led to the identification of districts prioritized

for elimination. A step-wedge, health facility catchment randomized design was used to identify areas for inaugural campaigns conducted in two Southern Province Districts (Gwembe and Sinazongwe) in December 2011 and January 2012. Community Health workers (CHW) were trained on standard procedures for screening, testing with malaria rapid diagnostic tests (RDT:SD Bioline Malaria A Pf) and treatment (artemether-lumefantrine) and, working collectively through catchment areas, performed a screening census to identify RDT positives and treat infections. Across 10 health facility catchments, 52,374 people were listed, 47,516 (91%) were tested and 12,961 (27%) were malaria RDT positive. Catchment teams achieved on average 9.8 households tested per day and completed catchment screenings on an average within 30 days. Across the catchments, RDT parasite prevalence ranged from 1.2% to 39.9%. Households on average reported 0.65 ITNs (range: 0.26-0.99). A cost per infected-case tested was estimated to be $7.21 and the cost per treated case estimated to be $8.06. In conclusion, next-in-line strategies for reducing community malaria transmission should involve optimizing vector control and population-wide malaria infection treatments which in combination have the potential to push communities toward malaria elimination. Subsequent rounds of these campaigns will be evaluated to assess their effectiveness in reducing malaria burden.

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MOBILE RAPID REPORTING SYSTEM IMPROVING MALARIA MONITORING IN ZAMBIA

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Insecticide-treated nets (ITNs) are an effective tool in controlling malaria and a primary malaria prevention strategy for the Malawi National Malaria Control Program (NMCP). In October 2010, the NMCP conducted a mass distribution of ITNs on Likoma Island on Lake Malawi to rapidly scale up prevention coverage and this was followed by a repeat mass ITN distribution during 2011. Two censuses were conducted one year apart, in October 2010 and in November 2011, to assess ITN coverage and malaria parasite prevalence levels among local residents using similar questionnaires and test methods. Children under age five were tested to determine anemia (Hemocue® HB 105) and malaria parasite prevalence using both slide microscopy and rapid diagnostics tests (RDTs: Clearview® Malaria PF). 11,079 and 10,923 household members were listed on Likoma Island in the 2010 and 2011 censuses, respectively. The percentage of households with at least one ITN increased from 64% to 84% from 2010 to 2011. The percentage of children under age five reporting to have slept under an ITN the previous night was 45% in 2010 and 42% in 2011; pregnant women who reported sleeping under an ITN the previous night was 36% in 2010 and 34% in 2011. Malaria related disease burden decreased over the year, with severe anaemia (Hb<7 gm/dl) in children falling from 7.0% in 2010 to 4.2% in 2011; overall anemia declined from 59% to 54% and malaria parasite prevalence among children decreased only slightly from 9.2% in 2010 to 7.8% in2011. Prior to 2010, ITN distributions in Likoma Island were through routine health facilities targeting only children and pregnant women and had achieved only low coverage. Leading up to the development of the National Malaria Strategic Plan 2011-2015, the repeated population wide distribution of ITNs in 2010 and 2011 achieved and then further increased high household ITN ownership. Based on the two full population assessments of Likoma Island residents after the ITN distributions, reported ITN use the previous night and malaria parasitemia rates showed little change while severe anemia in young children showed the largest relative decline of malaria related health outcomes. The NMCP and its supporting partners have continued to use population-wide distribution of ITNs, have begun innovative education and advocacy at community level to ensure proper use of ITNs, and are exploring additional options to further reduce malaria transmission on the Island.

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FOLLOW-UP CENSUS BASED PARASITE PREVALENCE ASSESSMENT AND IMPACT OF INSECTICIDE-TREATED NETS (ITN) COVERAGE ON LIKOMA ISLAND, MALAWI

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Insecticide-treated nets (ITNs) are an effective tool in controlling malaria and a primary malaria prevention strategy for the Malawi National Malaria Control Program (NMCP). In October 2010, the NMCP conducted a mass distribution of ITNs on Likoma Island on Lake Malawi to rapidly scale up prevention coverage and this was followed by a repeat mass ITN distribution during 2011. Two censuses were conducted one year apart, in October 2010 and in November 2011, to assess ITN coverage and malaria parasite prevalence levels among local residents using similar questionnaires and test methods. Children under age five were tested to determine anemia (Hemocue® HB 105) and malaria parasite prevalence using both slide microscopy and rapid diagnostics tests (RDTs: Clearview® Malaria PF). 11,079 and 10,923 household members were listed on Likoma Island in the 2010 and 2011 censuses, respectively. The percentage of households with at least one ITN increased from 64% to 84% from 2010 to 2011. The percentage of children under age five reporting to have slept under an ITN the previous night was 45% in 2010 and 42% in 2011; pregnant women who reported sleeping under an ITN the previous night was 36% in 2010 and 34% in 2011. Malaria related disease burden decreased over the year, with severe anaemia (Hb<7 gm/dl) in children falling from 7.0% in 2010 to 4.2% in 2011; overall anemia declined from 59% to 54% and malaria parasite prevalence among children decreased only slightly from 9.2% in 2010 to 7.8% in2011. Prior to 2010, ITN distributions in Likoma Island were through routine health facilities targeting only children and pregnant women and had achieved only low coverage. Leading up to the development of the National Malaria Strategic Plan 2011-2015, the repeated population wide distribution of ITNs in 2010 and 2011 achieved and then further increased high household ITN ownership. Based on the two full population assessments of Likoma Island residents after the ITN distributions, reported ITN use the previous night and malaria parasitemia rates showed little change while severe anemia in young children showed the largest relative decline of malaria related health outcomes. The NMCP and its supporting partners have continued to use population-wide distribution of ITNs, have begun innovative education and advocacy at community level to ensure proper use of ITNs, and are exploring additional options to further reduce malaria transmission on the Island.

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VARIANTS WITHIN THE FCΓRIIIA-176A/C AND TOLL-LIKE RECEPTOR (TLR)-9 (-1237T/C) GENE PROMOTER IS ASSOCIATED WITH INCREASED SUSCEPTIBILITY TO PEDIATRIC SEVERE MALARIAL ANEMIA AND CIRCULATING IFN-Γ

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Plasmodium falciparum malaria is still one of the leading global causes of infectious disease burden. In *P. falciparum* holoendemic transmission areas, such as in Siaya District in western Kenya, high rates of malaria-related pediatric morbidity and mortality is due to severe malarial anemia [SMA, hemoglobin (Hb)<6.0g/dL, any density parasitemia]. Since Toll-like receptors (TLRs) and Fc-γ receptors (FcγR) affect innate and adaptive immune responses, the functional association between polymorphic variants within FcγRIIIA (-176A/C, rs396991) and TLR-9 (-1237T/C, rs5743836) and susceptibility to SMA were investigated in children (n=301) presenting with acute malaria in a *P. falciparum* holoendemic transmission region in western Kenya. Hematological and parasitological

profiles were determined in all study participants. TaqMan 5′ allelic discrimination assay was used to determine FcγRIIIA-176A/C and TLR-9 -1237T/C genotypes. Circulating interferon (IFN)-γ levels were measured using Enzyme-Linked Immunosorbent Assay (ELISA). Multivariate logistic regression analyses controlling for potential confounders demonstrated that the presence of FcγRIIIA -176C/TLR-9 -1237T (-176C/-1237T) (OR; 2.05, 95% CI, 1.19-3.53; *P*=0.009) increased susceptibility to SMA two-fold while individuals -176C/-1237C were protected from SMA (OR; 0.36, 95% CI, 0.20-0.65; *P*=0.001). Consistently, stratification according to WHO definition of SMA (Hb<5.0g/dL, any density parasitemia) demonstrated that -176C/-1237T increased susceptibility (OR; 2.88, 95% CI, 1.61-5.17; *P*<0.0001) while -176C/-1237C protected from SMA (OR; 0.31, 95% CI, 0.14-0.68; *P*=0.004). Furthermore, the -176C/-1237C was associated with significantly higher circulating IFN-γ levels relative to non-176C/-1237C (*P*=0.014). Findings presented here demonstrate that variations in TLR-9 at -1237 and FcγRIIIA -176 are associated with increased susceptibility to SMA and functional changes in IFN-γ.

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VARIATION WITHIN THE INTERLEUKIN-13 (IL-13) GENE PROMOTER (-7402T/G AND -4729G/A) IS ASSOCIATED WITH SUSCEPTIBILITY TO PEDIATRIC SEVERE MALARIAL ANEMIA AND FUNCTIONAL CHANGES IN CIRCULATING IL-13

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Plasmodium falciparum malaria is still a major global cause of disease burden. In holoendemic *P. falciparum* transmission areas, such as western Kenya, severe malarial anemia [SMA, hemoglobin (Hb)<6.0g/dL, any density parasitemia] results in high rates of pediatric morbidity and mortality. Since interleukin-13 (IL-13) has been associated with pathogenesis of different infectious diseases, including *P. falciparum* malaria, the functional roles of polymorphic variants within IL-13 gene in conditioning susceptibility to SMA and reticulocyte production index (RPI; an effective measure of erythropoietic response in anemic children), were investigated. The relationship between the IL-13 variants -7402T/G (rs7719175) and -4729G/A (rs3091307) and susceptibility to SMA (Hb<6.0 g/dL, any density parasitemia) and RPI (<2) was investigated in children (n=387) with *falciparum* malaria from a *P. falciparum* holoendemic transmission region in western Kenya. Hematological and parasitological profiles were determined in all study participants. The IL-13 -7402T/G and -4729G/A genotypes were determined using TaqMan 5′ allele discrimination assay. Circulating IL-13 levels were measured using Enzyme-Linked Immunosorbent Assay. Multivariate logistic regression analyses controlling for potential confounders demonstrated that -7402G/-4729G (GG) (OR; 1.55, 95% CI, 1.01-2.37; *P*=0.044) were associated with increased susceptibility to SMA while -7402T/-4729A (TA) was associated with favorable erythropoietic response (RPI) (OR; 0.53, 95% CI, 0.29-0.98; *P*=0.043). In addition, carriers of the TA haplotype had significantly higher circulating IL-13 levels relative to non-TA haplotype (*P*=0.005). Findings presented here demonstrate that variation in IL-13 promoter at -7402T/G and -4729G/A is associated with increased susceptibility to SMA and functional changes in circulating IL-13 levels.

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A NEW MEMBER OF THE *PLASMODIUM VIVAX* TRYPTOPHAN RICH ANTIGEN (PVTRAG) MULTIGENE FAMILY SHOWS HIGH IMMUNOGENICITY AND RED BLOOD CELLS BINDING ACTIVITY

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Genes coding for the tryptophan-rich proteins are a part of multigene families identified in various *Plasmodium* species. Our recent studies have focused on the characterization of such antigens of the *P. vivax* parasite where we have investigated the possible role of these proteins in the elicitation of immunological responses and erythrocyte binding activity. Here, we report the immunological responses of a bacterially expressed recombinant and refolded His-fusion 32 kDa *P. vivax* tryptophan-rich antigen (PvTRAg32) amongst the *P. vivax* clinical isolates, its role in the erythrocyte binding activity, and its localization in the parasite. PvTRAg32 contains unusually high percentage of tryptophan residues (10.7%), which are positionally conserved with its orthologues in P. yoelii (PypAg1 and PypAg2) and *P. falciparum* (PfTryThrA and PfMATRA). Thirty four of the 40 (85.0%) *P. vivax* isolates showed seropositivity to recombinant PvTRAg32 by ELISA. The Mean ± SD values of OD for P.*vivax* subjects and naïve individuals were 1.02 ± 0.36 and 0.26 ± 0.11, respectively. In the Western blot analysis, majority of the subjects studied (n=44) showed high reactivity to the purified PvTRAg32. Immunoelectron microscopy and Immunofluorescence assays reveal PvTRAg32 is localized in micronemes and expressed at various erythrocytic stages of the parasite. Sequence analysis of the clinical isolates from various parts of the country shows that pvtrag32 is highly conserved and erythrocyte binding assays show significant binding as compared to the control antigens. High immunogenicity, conserved nature, and red cells binding activity could implicate this antigen as a multi subunit vaccine candidate against *P. vivax*.

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FILTER PAPER COLLECTION OF *PLASMODIUM FALCIPARUM* MRNA FOR DETECTING SUBMICROSCOPIC GAMETOCYTE DENSITIES

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Accurate sampling of submicroscopic gametocytes is essential for epidemiological studies to identify the infectious reservoir and enable appropriate application of disease control methods. Using gametocyte mRNA as a target enables sensitive detection, but requires careful handling of samples due to the labile nature of RNA. Filter papers can be used for collecting RNA samples but their capacity to withstand adverse storage conditions has not previously been rigorously tested, and evaluated with molecular detection methods QT-NASBA and RT-PCR in parallel. Three gametocyte dilutions; 10 g/µl, 1.0 g/µl and 0.1g/µl were spotted onto filter papers that were stored under; frozen, cold chain or tropical conditions for up to 3 months. RNA was extracted and detected by QT-NASBA and RT-PCR. Detection was higher from the Whatman 903 Protein Saver Card compared to the Whatman FTA Classic Card, by both techniques (p<0.0001). Storing papers at temperatures warmer than-80°C lead to a significant decrease in detection for the Whatman 903 Protein Saver Card, when evaluated by QT-NASBA p=0.025 and RT-PCR p=0.088.This study indicates that RNA can be recovered from filter papers, that the Whatman 903 Protein Saver Card is superior for this purpose to the Whatman FTA Classic Card for RNA sampling, but that storage above >-80°C will result in a loss in detection. Our findings indicate that in absence of optimal storage conditions, filter papers provide a useful alternative to low density gametocyte sampling.

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GENE EXPRESSION CHANGES AND SEQUENCE VARIATION IN *PLASMODIUM FALCIPARUM* CHLOROQUINE ADAPTED STRAINS

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The current scope of chemotherapy in the treatment of malaria has been limited due to the development of drug resistance to a number of

highly effective drugs including the use of chloroquine. Mutations in the *Plasmodium falciparum* gene loci Pfmdr1 N86Y and Pfcrt K76T are known to confer drug resistance to chloroquine. The role played by genetic mutations versus changes in gene expression levels and gene copy number has been highlighted, but the overall effect of each mechanism, singly or in interaction, to cause variable changes that mediate drug sensitivity still remains to be clearly defined. To determine the of role altered gene expression levels and gene copy number of the target loci Pfmdr1 and Pfcrt in mediating drug resistance in P. f. chloroquine adapted strains that is not associated with single nucleotide polymorphism gene mutations. P. f. strains adapted via cultures to different concentrations of chloroquine will be monitored for decreased drug sensitivity via the 3H-Hypoxanthine incorporation method at different drug exposure levels. SNPs primarily at Pfmdr1 86 and Pfcrt 76 will be determined via a novel Real Time-PCR allelic discrimination TaqMan genotyping assay and gene sequencing, respectively. Gene expression and copy number will be determined by a Real Time-PCR relative quantification assay. There is a significant change in the IC_{50} values of the *Plasmodium falciparum* strains beyond the drug exposure level equivalent to its IC_{30} an indicator of developing drug resistance. SNP analysis shows that the genotype of the strains primarily at Pfmdr1 86 (A, [N]) and Pfcrt 76 (A, [K]) remains unchanged over the course of the study in its wild type state. We next intend to look at changes in the level of gene expression and copy number, and compare its overall effect in the development of resistance to chloroquine versus SNP gene mutations. The course of drug resistance development in P. f. still remains highly uninvestigated as pertains to the specific manner and mechanisms by which it occurs. This may assist in understanding the molecular evolutionary mechanisms of anti-malarial drug resistance by linking genetic polymorphisms, gene expression levels and drug sensitivity and assisting in further monitoring and evaluation of malaria drug resistance in current treatment practices.

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THE HEME BIOSYNTHESIS PATHWAY IS NOT ESSENTIAL FOR *PLASMODIUM FALCIPARUM* DURING BLOOD-STAGE GROWTH

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Malaria parasites appear to have a functional heme biosynthesis pathway, although they potentially have access to the large amount of heme resulting from hemoglobin digestion in the digestive vacuole. They are believed not to salvage heme since almost all digestive heme is crystallized to form hemozoin. The generally accepted view in the field has been that the *de novo* heme synthesis pathway is essential for malaria parasites, and thus a good prospective drug target. Thus it came as a surprise that we were able to knock out genes for the first and the last enzymes, viz. 5-aminolevulinate synthetase (ALAS) and ferrochelatase (FC), of the malarial heme biosynthesis pathway. ALAS synthesizes 5-aminolevulinic acid from glycine and succinyl-CoA while FC inserts a ferrous iron into protoporphyrin IX to generate the final product, heme. Parasites of both knockout lines exhibited fully normal growth, suggesting the heme biosynthesis pathway is not essential or very important during the asexual blood stages. Since heme is the prosthetic group of several cytochromes (a-, b-, & c-types), which are components of the essential mitochondrial electron transport chain (mtETC), malaria parasites must apparently salvage heme, most likely from hemoglobin digestion. These results are remarkable because 1) they challenge the dogma that heme biosynthesis is essential in malaria parasites; 2) they support the idea that malaria parasites can also salvage heme; and 3) they may inform investigations involving antimalarial drug discovery. Further characterizations of these knockout parasites are underway to fully realize the implications of these unexpected findings.

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GENETIC MECHANISMS FOR AVIAN MALARIA HOST-SPECIFICITY

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A characteristic of emerging diseases is the switching of pathogens to novel hosts. In a time of global anthropogenic changes, it is important to study environmental influences on pathogen host-switching, and the genetic mechanisms responsible for host specificity in natural populations. While it is clear that habitat degradation can threaten bird populations, it is unclear how these alterations affect disease transmission and disease susceptibility. *Plasmodium*, the causative agent for malaria, has become an emerging disease in some organisms due to its ability to switch hosts. Avian malarias are unique because certain strains are host-specific; they can only infect one species of birds, but not another within the same family. While other strains are generalist; they can infect multiple species of birds. Furthermore, birds are inter- and transcontinental migrants and harbor well-characterized generalist and host-specific parasites. This makes them great models in research concerning parasite transmission and specificity. We aim to identify the genetic factors that contribute to and regulate the host-switching capabilities of pathogens, particularly in *Plasmodium* species that infect chicken and other birds. Specifically, we propose to characterize the erythrocyte binding-like family of genes extracellular binding domain region because they have been implicated in determining malaria host-specificity. We hypothesize that gene expression levels vary between generalist and specialist parasite, and that the genetic variations within the binding domain accounts for host-specificity and the spread of virulent, generalist malaria strains. Using high-throughput sequencing, we will profile the expression of mRNAs during *P. gallinaceum's* erythrocyte stage of infection. Our goal is to identify genes involved in host-specificity and determine the molecular differences that allow parasite transmission to a small or broad host range. This project will result in the first transcriptome, or analysis of total transcripts, of a malaria parasite infecting non-mammalian hosts. It will provide novel information on host-switching factors that shape pathogen virulence and threaten naive host populations.

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GENOME-WIDE VARIATION AND SELECTION SIGNATURES IN *PLASMODIUM FALCIPARUM* CLINICAL ISOLATES FROM A RURAL MALAWIAN POPULATION

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Implementation of large scale malaria interventions is bound to greatly disturb the natural environments of *Plasmodium falciparum*, by providing a selection landscape within which the parasite has to adapt. In this study, we have examined the resulting adaptive mechanisms to provide a map of genetic variation and signatures of selection from whole genome analysis of 89 uncultured *P. falciparum* clinical isolates from a Malawian population. We have catalogued SNPs, small insertions and deletions, and larger structural variants in our population. Our genome-wide analysis for selection signatures has revealed both known and novel vaccine and drug candidates. Understanding this variation against a background of intense malaria interventions will be key to controlling malaria and inform better use of the few interventions still available.

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ROLE OF POLYMORPHIC VARIATION IN *IL17* IN CONDITIONING THE CLINICAL OUTCOMES OF *PLASMODIUM FALCIPARUM* INFECTION IN AFRICAN CHILDREN

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In East Africa, children during the first two years of life are highly susceptible to severe malarial anemia [SMA, hemoglobin(Hb)<5.0g/dL]. We and others have found that genetic variation in innate immune response genes (IRGs) influences clinical outcomes in these children since their adaptive immune response has not fully developed. Among the IRGs, the role of interleukin (IL)-17, a pro-inflammatory cytokine responsible for neutrophil attraction to extracellular pathogens through anti-microbial peptide production, is largely unexplored in children with SMA. As such, we focused on several single nucleotide polymorphisms (SNP) in the *IL-17* promoter with a high minor allele frequency in the target population [-399A/G (rs3819024, A=0.68, G=0.32)and -1803T/C (rs677901, T=0.82, C=0.18)]. Multivariate logistic regression was performed in children (aged 3-36mos) infected with *Plasmodium falciparum* malaria (n=780) controlling for the confounding effects of age, gender, HIV-1 status, bacteremia, G6PD, α-thalassemia, and sickle-cell trait. Findings demonstrated that carriers of the -399G/-1803C haplotype had decreased susceptibility to SMA (OR=0.67, 95%CI=0.45-1.00, *P*=0.05). Additional analyses aimed at determining bone-marrow responses in the cohort revealed that the GT haplotype was associated with a lower reticulocyte production index (RPI <2, OR=2.22, 95%CI=1.01-4.87, *P*=0.05), while the AC haplotype was associated with a higher RPI (OR=0.54, CI 95%=0.36-0.79, *P*=0.002). Collectively, these results demonstrate that haplotypes in the *IL17* promoter condition susceptibility to SMA and reticulocyte production in this population.

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RELATIONSHIP BETWEEN HAPLOTYPES OF *IL13* PROMOTER POLYMORPHISMS AND MALARIA OUTCOME MEASURES IN KENYAN CHILDREN

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Plasmodium falciparum-associated severe malaria anemia (SMA) is a major cause of morbidity and mortality in sub-Saharan African children. The pathology of SMA is a consequence of the balance between pro- and anti-inflammatory mediators. Interleukin (IL)-13 is a powerful anti-inflammatory cytokine whose gene is located in a haplotypic block in the 5q31 cytokine gene cluster which also includes *IL3*, *IL4*, *IL5*, and *IL9*. We recently showed that the IL-13 pathway was associated with the development of SMA in Kenyan children. In addition, variations within the *IL13* promoter have been associated with several infectious diseases; including severe malaria among Thai adults. To further explore the role of IL-13 in malaria, we investigated the relationship between *IL13* -590A/G (rs2069743), -1257A/G (rs2069739), and -1456A/C (rs1881457) variants and malaria outcome measures (i.e., SMA and high-density parasitemia; HDP) among *falciparum* parasitemic children (n=850; aged 3-36 mos) presenting at the Siaya District Hospital, western Kenya. Children were dichotomized into either SMA (hemoglobin (Hb) <5.0g/dL; n=287] and non-SMA (Hb≥5.0g/dL; n=563), as well as HDP (HDP; ≥10,000 parasites/μL; n=573) and low-density parasitemia (LDP; <10,000 parasites/μL; n=277). Multivariate logistic regression model controlling for the confounding effects of age, gender, sickle-cell trait, HIV-1 and bacteremia status, showed no significant relationships between the haplotypes and SMA. However, the AGA haplotype was associated with a significantly increased risk of developing HDP (OR; 1.72, 95% CI 1.02-2.90, *P*=0.043), while carriage of the GAA haplotype was associated with significantly lower circulating IL-13 levels [median (IQR); 22.70 (34.56)] relative to individuals lacking the haplotype [28.45 (35.93); *P*=0.044]. Results presented here suggest that *IL13* variants may be important for conditioning susceptibility to parasitic burden, but not SMA, and underscore the complexity in discerning the role of the Th-2 cytokines in mediating malaria disease outcomes.

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EXTENDED HAPLOTYPES IN THE MIG (CXCL9) AND IP-10 (CXCL10) PROMOTERS INFLUENCE SUSCEPTIBILITY TO *PLASMODIUM FALCIPARUM*-ASSOCIATED SEVERE MALARIAL ANEMIA

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Plasmodium falciparum-induced severe malarial anemia [SMA, hemoglobin (Hb)<5.0g/dl] is a leading cause of morbidity and mortality in African children. However, the underlying genotypic traits that condition SMA have not been fully elucidated. Chemokines such as interferon gamma-induced protein-10 (IP-10/CXCL10) and monokine-induced by gamma (MIG/CXCL9) are typically increased during a type 1 inflammatory response. We recently observed that circulating levels of IP-10, MIG, and IFN-γ were significantly reduced in children with SMA compared to parasitized children with non-SMA (*P*=0.002, *P*=0.009, and *P*=0.019, respectively). To investigate the role of polymorphic variation in IP-10 and MIG on susceptibility to SMA, extended haplotypes were constructed using three promoter variants [IP-10 -1035G/A (rs4257674), IP-10 -1919G/T (rs4371639), and MIG -560G/A (rs6532083)] on chromosome 4. These particular variants were selected based on a high minor allelic distribution in the population. Multivariate logistic regression analysis (n=768; aged 3-36mos), controlling for covariates (age, gender, G6PD, HIV-1, bacteremia, α-thalassemia, and HbAS status) revealed that the ATA extended haplotype (-1035A/-1919T/-560A) was protective against SMA (OR:0.345, 95%CI: 0.225-0.530, *P*<0.001), and a favorable bone marrow response (RPI>2; OR:1.515, 95%CI: 1.045-2.197, *P*=0.028). In contrast, the extended GGG haplotype showed increased susceptibility to SMA (OR:4.087, 95%CI: 2.302-7.255, *P*<0.001) and decreased bone marrow responsiveness (OR:0.274, 95%CI: 0.138-0.543, *P*<0.001). Further analyses showed that carriage of the ATA haplotype was associated with increased circulating IP-10 levels (*P*=0.159), whereas carriage of the GGG haplotype was associated with reduced IP-10 (*P*=0.004) and MIG (*P*=0.156) levels. These results illustrate that variation in the IP-10/MIG extended haplotypes are associated with susceptibility to SMA and altered production of inflammatory mediators known to influence the clinical outcomes in children with malarial anemia.

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PEPTIDE NUCLEIC ACIDS (PNAS) AS A TOOL TO INVESTIGATE *PLASMODIUM FALCIPARUM* GENE FUNCTION

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Malaria remains a significant cause of morbidity and mortality worldwide. Drug resistance is widespread, and with a safe and effective vaccine still unavailable, new chemotherapeutic agents are required to ensure that cheap and effective treatment is widely available. To improve treatment options into the future we must improve our understanding of malaria parasite biology and identify new drug targets. A significant bottle-neck in the validation of targets for drug development has been the lack of robust methods to study gene/protein function, particularly when the gene of interest is essential to parasite growth and development. We have been investigating the use of Peptide Nucleic Acids (PNAs) as a tool to examine the function of proposed drug targets in *Plasmodium falciparum*. PNAs are DNA analogues that are assembled from a pseudo-peptide backbone. They bind to DNA and RNA via Watson-Crick base-pairing rules and as a consequence can change cellular gene expression. As PNAs lack a negatively-charged backbone their hybridisation to DNA/ RNA occurs without electrostatic repulsion and is stronger and faster than normal base pairing. Furthermore, PNAs are inert to natural proteases and nucleases and do not activate RNAase H. Our studies confirm that PNAs can be delivered into P. falciaprum asexual parasites and that these molecules can be used to investigate gene/protein function in *P. falciparum*.

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ESTIMATION OF WITHIN-HOST HAPLOTYPE FREQUENCIES OF *PLASMODIUM FALCIPARUM*

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The recent emergence of resistance to artemisinin derivatives reinforces the need for effective surveillance of antimalarial resistance. The spread of parasites that carry drug-resistant haplotypes from their point of origin is a major driver of antimalarial resistance. However, the accurate detection of the resistant haplotypes from patient samples is problematic: individuals can be infected with multiple parasite clones, especially in areas of high endemicity. When the multiplicity of infection (MOI) exceeds one, the allele sequences of the constituent clones at genotyped loci cannot be reconstructed. Consequently, statistical methods are required to deconvolute the allele sequences, infer distinct haplotypes and ascertain their frequencies from prevalence data. To address this problem, a Bayesian model for estimating haplotype frequencies has been developed. The model estimates haplotype frequencies based on prevalence data collected for one or more molecular markers known to been associated with antimalarial resistance. Prior knowledge of the MOI is not required. The model cycles over different, possible MOIs and, for each, calculates the likelihood of the data for potential estimates of the haplotype frequencies. The model quantifies the uncertainty in the frequency estimates using a Metropolis-Hasting Monte Carlo Markov chain algorithm. For each haplotype the model returns a distribution of frequency estimates from which the mean and its credible interval are derived and the average MOI for each sample. The model is then validated using simulated data sets for which the true haplotype estimates are known. We present results of the application of our algorithm and model to estimate haplotype frequencies for a set of historic data from Africa in which the prevalence of mutations associated with sulphadoxine-pyrimethamine resistance were obtained.

1196

MOLECULAR ANALYSIS OF *PLASMODIUM FALCIPARUM* INFECTIONS FROM CORD BLOOD SAMPLES OF NEWBORNS IN RURAL NORTHEASTERN GHANA

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Umbilical cord blood samples collected as part of a large (n = 2097) birth cohort study in rural northeastern Ghana were assayed by PCR to identify placental malaria infections and assess the impact of sulfadoxine/ pyrimethamine (SP)-based intermittent preventive treatment during pregnancy (IPTp). Cord blood *Plamodium falciparum* infections were 38% more prevalent in first time pregnancies and the IPTp target of 3 SP doses in the last six months of pregnancy was associated with 36% fewer cord blood infections in newborns. We hypothesized that: 1) malaria infections in births by primigravidae would reflect a greater clonal composition, owing to abundant available binding sites for *P. falciparum* in the placenta of first time pregnancies, and 2) *P. falciparum* infections in deliveries by mothers who did use SP would carry a higher frequency of point mutations associated with SP resistance. We analyzed five microsatellite markers from cord blood positive deliveries by primigravid and multigravid mothers that were matched for S/P doses and bednet (ITN) use. Tandem repeat polymorphisms in cord blood positives were determined by amplification of fluorescent-labeled PCR products which were then sequenced. Positive cord bloods were analyzed for single nucleotide polymorphisms (SNPs) in *P. falciparum* genes associated with drug resistance using a multiplexed microsphere-based suspension array platform. These analyses included dihydrofolate reductase (*Pfdhfr*) and dihydropteroate synthase (*Pfdhps*), key markers of sulfadoxine/ pyrimethamine (S/P) resistance, and the multidrug resistance protein 1 (*Pfmdr1*) and chloroquine resistance transporter (*Pfcrt*) genes. Mutation frequencies detected in *P. falciparum* from cord blood infections are being compared and analyzed against maternal doses of SP taken for IPTp and against a *P. falciparum* profile from this same location in 2001, before chloroquine was replaced by artesunate-amodiaquine, as the standard for uncomplicated malaria treatment and before SP became the standard for malaria prevention in pregnancy.

1197

A MORE ANCIENT ORIGIN FOR HIGH COPY NUMBER OF THE MEROZOITE SURFACE PROTEIN 3 (MSP3) FAMILY

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Gene families encoding antigens have been shown to be actors in many key processes of host cell invasion and immune evasion for the *Plasmodium* parasites that cause malaria. Lineage specific differences in gene copy number for these families are thought to be evidence of adaptation with consequences for pathogenesis, however these

differences are often elucidated from few or distantly related species. The ecological ubiquity of *Plasmodium* species, along with a growing richness of genomic data, allows more accurate insights about the evolution of gene families and adaptation. Previous comparisons hypothesized a *P. vivax* lineage specific expansion of the Merozoite Surface Protein 3 (MSP3) family. We show here that the macaque parasite P. cynomolgi, as well as other related species, vary in copy number and that some species exhibit copy number equivalent to that of *P. vivax*. This indicates that high copy number alone in the MSP3 array is not a result of adaptation to the human host by *P. vivax*, and predates its common ancestor with P. cynomolgi (estimated 2.36-5.27 mya). In addition, phylogenetic inference and synteny indicates that the MSP3 family as described for *P. falciparum* may be analogous rather than homologous to that of *P. vivax* and its related species. Variable patterns of genetic diversity, domain architecture and protein similarity ranging from 18 to 80% were observed among the putative *P. vivax* MSP3 members, suggestive of diversifying selection acting to drive the divergence of paralogs or reflecting gene duplication events at multiple time scales. High levels of silent and replacement polymorphisms between P. cynomolgi and *P. vivax* MSP3 genes point to rapid divergence of the family between even the closest related species. Our findings caution against the inference of adaptation based on copy number changes alone, and show the utility of placing lineage specific gene family changes in an evolutionary context, especially when the signal of homology is low.

1198

PFF1320C, A PUTATIVE MYOSIN LIGHT CHAIN OF *PLASMODIUM*

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Little is known regarding the nature and function of the myosin motors of *Plasmodium*. Analysis of the genome reveals that there are six myosin heavy chains but only the role of Myosin A, along with its cognate light chain partner, myosin A tail interacting protein (MTIP), has been well characterized in the process of parasite invasion. To date, only MTIP has been characterized as a *Plasmodium* myosin light chain. We have begun characterizing an annotated putative myosin light chain, however there is no biochemical evidence suggesting that the molecule functions in this manner. PFF1320c has been conserved within the primate infecting plasmodia, *P. vivax* and *P. knowlesi,* in addition to *P. falciparum*, yet this protein has been evolutionarily lost in the rodent parasites, *P. berghei*, *P. yoelii* and *P. chaubaudi*. Interestingly, in *P. falciparum* this protein appears essential due to our inability to disrupt the gene. However, upon expression of an HA tagged version of the gene at an ectopic site, we were able to knock out the gene. Preliminary studies have shown that this presumptive myosin light chain assembles in a high molecular weight complex indicative of formation of a myosin motor. Further studies are currently underway to identify the myosin heavy chain partner, determine the subcellular localization of this myosin motor and to characterize the biological role of this complex.

1199

A COMPARISON OF FOUR MOLECULAR ASSAYS FOR *PLASMODIUM* MALARIA PARASITES REVEALS FALSE POSITIVES AND FALSE NEGATIVES IN *ANOPHELES* VECTORS

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The detection of *Plasmodium* malaria parasites in *Anopheles* vectors is critical for understanding malarial transmission dynamics and for incriminating the vector species. Several PCR based methods have been developed for *Plasmodium* in human blood, but less so in the vector. The *18S rDNA* locus is the most commonly used molecular target although more multi-copy loci have recently been examined. We compared four *Plasmodium* specific molecular assays using field collected *Anopheles* mosquitoes from the Republic of Korea, and laboratory infected *An. stephensi*. The assays included: 1) a nested PCR technique targeting *18S rDNA* that has been previously used to test vectors, UNR/VIV; 2) a second commonly used *18S rDNA* assay, PLU/VIV; 3) a multi-copy nuclear marker, Pvr47; and 4) a nested PCR targeting the *Cytochrome B,* mitochondrial marker, *CytB*. Amplicon from PCR was sequenced and compared to known *Plasmodium spp*. in the *Plasmodium* Genome Resource (www.PlasmoDB.org). The specimens identified as positive, and the positivity rates, differed according to the assay. Two of the PCR assays, UNR/VIV and Pvr47*,* produced multiple banding patterns. DNA sequences for 3 of 4 PCR assays from the field collected specimens matched to non-*Plasmodium* organisms, which included the arthropod fungus, *Zoopthera sp.*. The nested *CytB* assay produced unequivocal results; detecting only one individual infected with *P. bergheii.* The *CytB* and UNR/VIV assays were then compared using *P. falciparum* infected *An. stephensi* incubated for different times post infection to measure sporozoite and oocyst detection rates. The *CytB* assay was more sensitive than the *18S rDNA* assay, detecting more infections of oocysts in the abdomen and of sporozoites in the salivary glands. We found that two commonly used primers for detecting *Plasmodium* targeting the *18S rDNA* region produces apparent false positives and negatives when applied to field-collected mosquito stage parasites. We recommend the use of the *CytB* assay to detect *Plasmodium spp*. in field studies, which aim to measure mosquito stage parasite rates.

1200

BLOOD FILMS AS DNA SOURCE FOR MOLECULAR DIAGNOSTIC OF MALARIA

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The use of molecular tools for malaria diagnostics has been spread successfully around the world; but its sensibility and specificity depends on the sample source and the quantity/quality of the DNA obtained. In order to explore other sources of parasite DNA, this study try to test if sufficient DNA could be successfully extracted from stained malaria blood films. One cultured blood sample with high parasitaemia (5%) was serially diluted in human whole blood, and 5 µL of each dilution was blotted on films in quadruplicates; then each film was stained with giemsa. Thus, from the four films of each parasitemia, two of them were extracted with Phenol-Chloroform and the other two with QIA amp DNA mini kit. The final elution step was made in 50 µL of buffer TE or 50uL of buffer AE respectively. As a positive control, the whole blood of each dilution was extracted with Phenol-Chloroform and QIA amp mini kit in duplicate. A total of 5 negative controls were used in order to detect cross contamination during the extraction process. The extracted DNA was amplified using a nested PCR method for *Plasmodium* species detection using 18S ribosomal gene as target. The DNA from blood films was successfully extracted with both, Phenol-Chloroform and QIA amp mini kit, methods. The detection limit of the nested PCR using DNA from stained blood films was 0.0016% of parasitemia compared to 0.00032% for the whole blood. None of the negatives controls amplified, showing that there were no cross contaminations during the extraction process. In conclusion blood films can be a good source of DNA to detect *Plasmodium* species by PCR without any problem of contamination, and the DNA from blood films combined with PCR could be used as a quality control for field diagnostic, or others genetics or molecular studies where other source of sample is difficult to obtain.

1201

UTILIZING DIRECT PATIENT SAMPLES FOR ANTIMALARIAL RESISTANCE GWAS IN *PLASMODIUM FALCIPARUM*

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Plasmodium falciparum malaria's rapid adaptation to new drugs allows it to remain one of the most devastating infectious diseases of humans. Understanding the genetic basis of these adaptations is critical to successful intervention. Association studies are an established tool for discovering the genetic mechanisms contributing to variation in drug responses. However, recent malaria-based GWAS have either had limited sample sizes due to the laborious nature of parasite culture adaptation or limited phenotype heritability due to the use of clinical phenotypes when avoiding culture adaptation. Here, we present a GWAS based on direct patient samples that utilizes *ex vivo* drug phenotypes and whole genome sequence of *P. falciparum*-enriched DNA using a hybrid-selection technique. This approach avoids both culture adaptation and clinical phenotypes and simplifies the process of performing a well-powered GWAS. We test 85 recently isolated parasites from Senegal against 8 antimalarial drugs_including amodiaquine, artemisinin, artesunate, dihydroartemisinin, chloroquine, pyrimethamine, mefloquine, and quinine. We adapt recent mixed-model GWAS tools, such as EMMA and GCTA, and selection tools, such as iHS and XP-EHH, to study the heritability of drug response phenotypes and identify known and novel loci associated with drug resistance at genome-wide significance. Additionally, we examine elements of population and genome structure and compare these to previously available sequence data from culture-adapted parasites. This demonstrates a highly scalable type of GWAS for antimalarial response, one that does not require time-intensive culture adaptation processes yet still utilizes an *ex vivo* drug phenotype that is less influenced by host or environmental factors than clinical phenotypes.

1202

CLINICAL AND PARASITOLOGICAL EVALUATION OF THE COMPARATIVE EFFICACY AND EFFECTIVENESS OF ARTEMETHER-LUMEFANTRINE, ARTESUNATE-AMODIAQUINE AND ARTESUNATE-AMODIAQUINE PLUS CHLORPHENIRAMINE IN NIGERIAN CHILDREN WITH ACUTE UNCOMPLICATED MALARIA

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Drug resistance has posed a serious threat to malaria chemotherapy in the past two decades. In order to curb this problem, WHO has recommended the use of artemisinin-based combination therapy (ACT) for the treatment of *falciparum* malaria. In Nigeria, artemether-lumefantrine (AL) and artesunate-amodiaquine (ASAQ) have been adopted as preferred options. However, ASAQ was only available as separate drugs that were co-administered until recently. Also chlorpheniramine has been reported to enhance the efficacy of amodiaquine. To encourage compliance, fixed dose combinations are recommended. Artemoclo™ (ATC), a fixed dose formulation of artesunate plus amodiaquine plus chlorpheniramine was evaluated for its comparative efficacy and effectiveness with AL and ASAQ for acute uncomplicated malaria in Nigerian children. Little is known about the therapeutic efficacy of these artemisinin combinations and the prevalence of molecular markers associated with antimalarial drug resistance. A total of 200 children with *Plasmodium falciparum* infection were recruited and randomized into three study groups (AL, ASAQ, and ATC). All patients were followed up for 42 days to study the clinical and parasitological responses according to the WHO protocol (2009). We assessed the polymorphism of the *pfcrt* and *pfmdr1*genes by direct sequencing method. Results will be presented here.

1203

THE EFFECT OF STORAGE CONDITIONS AND DNA EXTRACTION METHODS ON THE MOLECULAR ANALYSIS OF *PLASMODIA* FROM DRIED BLOOD SPOTS

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Extraction and amplification of parasite DNA from dried blood spots (DBS) has been widely used for the detection and genotyping of malaria parasites. However, there have been few systematic efforts to quantify the impact of DNA degradation on molecular assays. Using field samples spanning over a decade and well-characterized laboratory controls, we are evaluating the effects of duration of storage, storage conditions, and DNA extraction methods for DBS on 4 different PCR protocols: 1) nested PCR of 18S rDNA, 2) nested PCR of cytochrome B, 3) qPCR, and 4) single-round PCR of microsatellites. To date, we have completed evaluation of the effect of storage temperature and DNA extraction technique (saponin/Chelex vs. Qiagen spin column) for DBS containing known densities of laboratory-strain *P. falciparum* parasites (factors of 10 ranging from 1-10,000 parasites(p)/µl). Identically prepared samples were spotted on Whatman 903 filter paper, stored at -20C and ambient temperature for 2 years, than evaluated by PCR of 18S rDNA and 2 microsatellites. Samples stored at -20C visibly lysed more effectively during extraction. These samples were the only ones that were detected at 1p/µl, amplified more consistently (62.5% vs. 12.5% of samples) at ≤10p/µL by 18S PCR, and more robustly (2.4-4.7 fold higher peak intensities) by microsatellite PCR. A similar degree of increased sensitivity was seen comparing saponin/Chelex to spin column extraction yielding 62.5% vs. 12.5% positive respectively at ≤10p/µl by 18S PCR and a comparable increase in intensity (2.1-4.7 fold) by microsatellite PCR. The samples that were stored at -20C and Chelex extracted were detected at all parasite densities consistently (100% sensitivity) in 18S PCR and showed the highest relative concentration (4.7 fold higher compared to ambient column samples) of parasite DNA by microsatellite PCR. These data suggest that storage of DBS at -20C and extraction using the saponin/Chelex method provide greater sensitivity for detection of plasmodial DNA. More extensive analysis of field isolates is in process.

1204

CO-OCCURRENCE AND DISTRIBUTION OF EAST (L1014S) AND WEST (L1014F) AFRICAN KNOCKDOWN RESISTANCE MUTATIONS IN *ANOPHELES GAMBIAE S.L.* IN TANZANIA

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The use of insecticides has remained the principal component of malaria control. However, the development of resistance by mosquito vectors to insecticides recommended for IRS and/or LLINs would potentially affect the gains so far achieved in malaria control. The phenotypic data of the current national surveillance on malaria vectors susceptibility to insecticides clearly indicates that there is a notable increase in vector tolerance and/or resistance to pyrethroid insecticides in Tanzania. Two mutations in the sodium channel, *1014F* and *1014S* are known to cause target site resistance to pyrethroids and DDT. These mutations are often referred to as the West and East African knock down resistance (*kdr)* mutations, respectively. We screened and characterized the *kdr* mutations in *Anopheles gambiae s.l.* mosquitoes sampled from 14 sentinel districts in Tanzania. Following insecticide susceptibility tests of wild vector populations, presence and typing of the *kdr* genotypes were determined in *An. gambiae s.l* using allele-specific polymerase chain reaction tests. The East African *kdr* allele was found to be present in nine specimens out of 160 with a frequency ranging from 4% to 20%. The West African *kdr* allele in heterozygous form was found in 30 specimens out of 160 with a frequency ranging from 12% to 52%. The East African *kdr* mutation was detected in three sentinel districts of Ilala, Muleba and Handeni whereas the heterozygous form of West African *kdr* mutation was detected in Muleba, Babati, Mvomero and Ilala. This observation is supported by the phenotypic data which indicates increased levels of pyrethroid resistance in a number of vector populations sampled. This situation calls for an urgent implementation of national mitigation measures and rational resistance management strategies in the country, if the gains so far made in malaria control are to be sustained.

1205

CHARACTERIZATION OF INSECTICIDE RESISTANCES OF *CULEX QUINQUEFASCIATUS*, *ANOPHELES GAMBIAE*, *AEDES AEGYPTI* AND *AE. ALBOPICTUS* IN MAYOTTE (FRENCH COMOROS ISLAND)

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Several vector-borne diseases have affected and still affect Mayotte Island, the main ones being malaria, chikungunya, dengue, Rift Valley fever and filariasis. Since 1950s, insecticides are used against mosquitoes vectors of these diseases. Organochlorines (OC), organophosphates (OP), pyrethroids (PYR) and, more recently *Bti* (bacterial toxin), generate strong selection pressures on the field mosquitoes populations, but no data on the resistance status of mosquitoes vectors was available until now. Bioassays carried out on larval and adult field populations show strong resistance of *Culex quinquefasciatus* to temephos (OP), dieldrin (OC) and deltamethrin (PYR), and temephos resistance in *Anopheles gambiae*. However, *Aedes aegypti* and *Ae. albopictus* remain susceptible to these insecticides. Biochemical assays for various generalists detoxifying enzymes (metabolic resistance) showed a significantly higher esterase and glutanione-S-transferase activities, but a lower oxidase expression in the field population of *Cx. quinquefasciatus* compared to the susceptible reference strain. Esterases overactivity appears to be linked to the superlocus *Ester*. In fact, the *Ester*^{2} allele was present on the whole island. In *An. gambiae* an esterase overactivity was also observed. Other resistance mechanisms due to insecticide target site modifications were investigated. The *ace-1*^{R} allele, coding for an insensitive acetylcholinesterase (OP target), is present in the ten tested populations. The *kdr* mutation, associated with deltamethrin resistance, is almost fixed on the entire island in *Cx. quinquefasciatus*. The *rdl* mutation of the GABA receptor (dieldrin resistance) is also frequent. In *An. gambiae*, the *ace-1*^{R} allele was not present in the resistant population, so that temephos resistance in this species seems to be due only to overexpression of detoxification mechanisms. In a context of drastic reduction of the range of available insecticides in Mayotte, the presence of both metabolic and target site resistances in two of the main vectors of the island is particularly worrying. A better understanding of the resistance mechanisms involved is essential to implement more efficient and sustainable control strategies.

1206

WHAT IS THE VALUE OF 'INCREASED EFFICACY' FOR NEW VECTOR CONTROL TOOLS?

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Significant progress in malaria control in the past decade can be attributed largely to the massive scale-up of insecticide-based vector control interventions, such as long-lasting insecticidal nets (LLINs) or indoor residual spraying of insecticides (IRS). To date, few studies have assessed the impact of insecticide resistance on malaria control, although there are reports of the reduced efficacy of pyrethroid treated mosquito nets and pyrethroid based IRS from several countries across Africa. Monitoring is a critical component of vector control, and adequate resistance data should be collected and used to inform the choice of vector control tools. Clearly tools with the highest efficacy are preferable, particularly in areas of pyrethroid resistance. Knowledge of the type and level of resistance present in mosquito populations is critical for the interpretation and communication of field-derived data. Different laboratory and field-based methods used to assess the efficacy of vector control tools will be discussed, using recent examples from studies conducted across Africa. The use of models to explore the predicted community-level and health impact of new tools will also be discussed to highlight the importance of maintaining a high level of efficacy, especially in the presence of insecticide resistance.

1207

INDOOR RESIDUAL SPRAYING FOR DENGUE CONTROL: A FIELD EXPERIMENT IN PENANG, MALAYSIA

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Indoor residual spraying (IRS) is not used routinely for control of dengue vectors, and the method is not recommended by WHO for this purpose. However, there is evidence in the literature that where IRS was used for malaria control, *Aedes aegypti* populations were reduced or eliminated even though they were not the target species. The effectiveness of two residually-sprayed insecticides, Icon 10CS (pyrethroid; lambdacyhalothrin) and Actellic (organophosphate; pirimiphos methyl) was evaluated for control of dengue vectors in a small community of traditional housing in a

single location in Penang, Malaysia. Notably, a number of confirmed cases of dengue had been recorded in theis community in the weeks prior to the start of the study. The impact on peridomestic populations of *Aedes aegypti* and *Ae. albopictus* was measured using the standard *Stegomyia* and pupal indices. As impact on nuisance mosquitoes is important for any domestic treatment, *Culex quinquefasciatus* infestations were monitored using CDC miniature light traps, placed in a room overnight with human sleepers that were protected by untreated bednets. Shortly after the baseline study, the intervention began in January 2012. Initial analyses at the first follow-up survey demonstrated that indices fell immediately following treatment but that there were no differences between treatments. The trial is ongoing but final results will be presented and prospects for application of IRS for control of dengue and chikungunya vectors discussed.

1208

MATING COMPETITIVENESS OF RIDL® MOSQUITOES AGAINST WILD TYPE MOSQUITO STRAINS FROM DIFFERENT GLOBAL REGIONS

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The use of sterile insects as a control method for insects requires that laboratory-produced sterile males released in the field out-compete wild males in the insemination of wild females. The threshold for this competitiveness for male Mediterranean fruit flies sterilized by irradiation occurs when 20% of the females are successfully mated by sterile males while competing with an equal number of wild type (WT) males. A field trial using male *Aedes aegypti* mosquitoes carrying a dominant lethal (RIDL®) gene that was developed by Oxitec, has been proposed to be conducted in Key West, Florida. To assess the competitiveness of the RIDL males against *Ae. aegypti* males from Key West, MRFU is measuring the mating competitiveness in semi-field cages of RIDL males against *Ae. aegypti* males reared from eggs recently collected in Key West. This poster presents results from MFRU and a series of small cage and large cage, laboratory and semi-field mating competitiveness studies that have been performed on RIDL strains of *Ae. aegypti* and *Ae. albopictus* who competed against WT laboratory strains of these species in several laboratories around the world. These results clearly show that RIDL strains of mosquitoes can compete successfully with WT males in inseminating females. If RIDL males were equivalent to wild type, then, in these tests on average 50% of the wild females would have been mated by a RIDL male, while, in fact, we observed 48% of the mating was made by RIDL males. This is several times the minimum requirement for male competitiveness observed in a successful fruit fly control program, and extremely encouraging for the use of this control intervention with mosquitoes.

1209

ANOPHELES GAMBIAE SEMINAL TRANSGLUTAMINASE AND *IN VITRO* CROSSLINKING OF ITS NATIVE SUBSTRATE PLUGIN

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Male Anopheline mosquitoes coagulate their seminal fluids via cross-linking of a substrate, called Plugin, by a specific seminal transglutaminase. Successful transfer of the 'mating plug' produced by the crosslinking of Plugin is necessary for efficient sperm storage by females. Here, we report the expression and purification of both Plugin and its transglutaminase, and *in vitro* reconstitution of the crosslinking reaction. Full-length *Anopheles gambiae* seminal transglutaminase is monomeric and of mixed a/b character as predicted by sequence homology to other transglutaminases. The C-terminal domain of Plugin is monomeric and of pronounced a-helical character, but is highly extended in solution and aggregates in solution with increasing concentration. Ca2+-dependent crosslinking of Plugin by *A. gambiae* seminal transglutaminase occurs readily *in vitro*. Tryptic digestion and mass spectrometric analysis of crosslinked Plugin identified several crosslinking sites present in the majority of a-helices in the C-terminal domain. Inhibition of the seminal transglutaminase, thereby preventing formation of the mating plug, may represent a potential specific and effective chemosterilant for *A. gambiae*.

1210

NOVEL GENETIC MARKERS FOR INSECTICIDE RESISTANCE IN EAST AFRICAN *ANOPHELES GAMBIAE*

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Resistance to pyrethroid insecticides is increasing in East Africa and in some areas now approaches the high levels documented in many West African populations of *Anopheles gambiae s.s.*. Both metabolic and target site resistance-mechanisms have long been implicated for East African populations, but, apart from a single target site mutation (*kdr L1014S*) DNA polymorphisms associated with pyrethroid resistance in field populations have not been identified. To investigate the genetic determinants of resistance in *An. gambiae* from an area of high pyrethroid resistance near the Uganda-Kenyan border, we performed association studies for class I and II pyrethroids in two consecutive years. Families established directly from the wild that differed in resistance levels were screened using several methodologies ranging from low-density candidate SNP arrays to whole genome resequencing and expression microarrays. Few of the genes significantly overexpressed in resistant compared to susceptible families were from detoxification gene families, and levels of overexpression were low, suggesting that up-regulation of few key metabolic enzymes is not the major factor underpinning resistance. Using independent screening technologies, significantly associated SNPs from genotyping and resequencing were tested further for association in samples from other locations. Repeatable associations were obtained for several SNPs, most notably exonic variants in carboxylesterase and P450 genes. To our knowledge these are the first field-replicated markers for metabolic insecticide resistance in *An. gambiae*, and we have designed Taqman assays to facilitate routine diagnostic screening alongside *kdr* mutations.

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PHOTOLARVICIDAL DIET FORMULATIONS OF A PORPHYRIN DERIVATE TO POTENTIATE MALARIA VECTOR CONTROL

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Photolarvicidal approach based on the use of a photo-sensitizer associated to a suitable larval diet is a new strategy to potentiate the control of vector populations resistant to conventional insecticides. This study aimed to screen and to evaluate the photolarvicidal efficacy of two candidate diet formulations of a porphyrin photo-sensitizer derivate (C12 porphyrin) on *Anopheles gambiae* larvae in a semi-field situation in Burkina Faso. Powdered cat food pellet and pollen from several families of plants were used as candidate larval diets and were separately incubated in a series of C12 porphyrin solutions ranging from 3.110-3-2mM in order to obtain

final dried powders of sunlight-activatable diets. In a first dose-efficacy assay, an optimal effective dosage of each candidate diet was assessed using *An. gambiae* Kisumu strain maintained in two different samples of water. In a second assay, the efficacy of an optimal effective dosage of each candidate diet complex was offered to three different colonies of *An. gambiae* larvae maintained in natural larval breeding water originated from four different localities of western Burkina Faso. C12 porphyrin free food samples were used for control treatment. After overnight feeding in the darkness, larvae were exposed under natural light (sunlight) to be irradiated and their mortality was timely recorded. More than 79.79 ± 23.36 % of larval mortality was obtained whatever the C12 porphyrin-candidate diet. However, the mortality varied significantly according to the type of diet and the source of natural larval breeding water ($p<0.05$) with the highest larval mortality (100%) caused by the fine fraction of Cat food formulate. These results suggest that these sunlight-activatable C12 porphyrin-diet complexes should be considered as new environmental friendly tool which could be efficacy and cost effective against *An. gambiae* larvae. However more extended studies, need to be performed in different ecological set-ups to better validate the field efficacy of such formulations.

1212

BEHAVIOR OF MALARIA VECTORS AT THE BEDNET INTERFACE

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The management of insecticide resistance in African malaria vectors is entering a critical era and the need to identify novel approaches for controlling insecticide resistant populations is now more urgent than ever. Given our limited knowledge at present, any increase in understanding of vector behavior has the potential to improve the design of new tools or approaches for vector control as well as broadening our understanding of the modes of action and mechanisms of resistance to different insecticides. Here we report on the initial findings of a study to characterize behavior of *Anopheles gambiae s.s.* at key stages during host location and bloodfeeding, and to investigate how insecticides impinge on these basic responses. The nocturnal activity of free-flying individual or groups of mosquitoes in the laboratory and in the field is video recorded in the absence of visible light, and analyzed subsequently. Sequences of distinguishable behavioral events have been defined and quantified. As recordings demonstrate, these quantifiable events provide a standard against which variations in field populations can be evaluated. The impact on these behaviors of different insecticide treatments has been evaluated and results will be presented. Studies aim also to quantify the responses of pyrethroid resistant vector populations using these systems, and to develop more advanced systems to track and characterize the behavioral events at other stages in the process of hostseeking. Investigations are ongoing but the findings of work in progress that has been largely completed will be presented, and potential application to vector control will be discussed.

1213

EVALUATION OF NOVEL SYNTHETIC MOSQUITOCIDES FOR CONTROL OF *AEDES AEGYPTI* AND *ANOPHELES GAMBIAE*

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There are many diseases that are transmitted by arthropods, and mosquitoes vector a number of them, such as dengue fever transmitted by *Aedes aegypti* and malaria by *Anopheles gambiae*. Current control programs include indoor residual spraying, and the use of insecticide-treated nets. With the advent of mosquito resistance, there is an urgent need for new control measures, including new insecticides with novel modes of action. With this in mind, we have been screening unique compounds in both *An. gambiae* and *Ae. aegypti* in adult lethality bioassays and larval paralysis assays. In these studies, neurotoxicants, such as tetraethylammonium (TEA) and 4-aminopyridine were shown to be paralytic to larvae at low ppm levels. A number of novel catechols were used in the adult assays, which showed some promising results, and with equal kill on the resistant Akron strain of *An. gambiae*. It is our expectation that through novel chemistry and molecular design, we will find a compopund that will be effective in mosquitoes while at the same time have little effect on humans, providing a safe and effective class of new insecticide.

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PILOT TRIAL TO DEVELOP MOSQUITOCIDAL IMMUNITY IN CATTLE

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This project explored the possibility of producing mosquitocidal immunity in cattle as a way to reduce malaria transmission in regions where the local vectors are exophagic and zoophilic. Groups of 24 male Holstein calves were injected intramuscularly with either saline (control) or with bacteria-free *Anopheles stephensi* midgut homogenates taken from either unengorged or from blood-engorged mosquitoes. Two types of adjuvants (TiterMax and ISCOM) were used. Calves received 2 injections spaced 2 weeks apart. After the second immunization, nearly all calves immunized against midgut preparations had anti-midgut antibody titers >1:100,000. There was no noticeable difference in antibody response due to the different adjuvants. Preimmune sera and sera from saline-injected control calves had no anti-midgut antibodies. To test for mosquitocidal activity, ca. 150 mosquitoes each were loaded into feeding cassettes fashioned from Tupperware containers, duct tape and dental dam. Cassettes were strapped with plastic wrap to the shaved sides of calves for 20-30 minutes and then returned to the insectary where mosquitoes were released into cubic foot screened cages. Unfed mosquitoes were removed. Mosquitoes were checked daily and dead mosquitoes were counted and removed. Some (ca. 50) mosquitoes were transferred into smaller cages and allowed to oviposit. Eggs were counted and the average number of eggs laid per mosquito was calculated for each group. Vaccination with the various mosquito midgut preparations and adjuvants produced only a transitory and/or modest mosquitocidal effect in some of the immunized animals. None of the immunized calves reduced mosquito fecundity. The mosquitocidal effect in this trial was much less pronounced than the robust acaricidal effects seen in early anti-tick vaccine trials where calves were similarly immunized with homogenates of tick midguts. Neverthess, the fact that even a modest mosquitocidal effect was achieved indicates that efficacious mosquitocidal vaccines in livestock are possible.

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MALARIA VECTOR BIONOMICS AND HUMAN MALARIA INFECTION RATES IN NCHELENGE, ZAMBIA

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As part of the International Centers of Excellence in Malaria Research (ICEMR) project, mosquito collections and human malaria screenings were conducted from March to April 2012 in Nchelenge District in Luapula Province, Zambia. Nchelenge experiences hyperendemic malaria despite continued implementation of indoor residual spraying (IRS) and long-lasting insecticide nets (LLIN) as control measures. Center for

Disease Control light traps (CDC LT) and pyrethroid spray catch (PSC) collections from 58 households in Nchelenge revealed the presence of both *Anopheles gambiae* s.s. and *An. funestus* s.s. 1287 mosquitoes were collected and identified morphologically. *Anopheles* and Culicines made up approximately 70% and 30% of the total mosquito collection respectively. Female anophelines made up 53% of the total mosquito collection; *An. gambiae* s.s. and *An. funestus* s.s. represented 13% and 87% of the collection respectively. This indicates that *An. funestus* s.s. is the dominant malaria vector with some contribution from *An. gambiae* s.s. The abundance of *An. funestus s.s.* and the high human malaria infection rates in Nchelenge support the hypothesis of high anthropophilic behavior and high sporozoite infection rates in *An. funestus* s.s. Accordingly, it is predicted that the human biting index, human biting rate, and entomological inoculation rate for *An. funestus* s.s. is higher than that of *An. gambiae* s.s. The multiple blood feeding behavior of both malaria vectors will also be explored to identify heterogeneity in biting. The vector data in Nchelenge present unique opportunities to further our understanding of malaria transmission and the implications for malaria control in high-risk areas.

1216

ILLUMINATION PREFERENCE OF *ANOPHELES GAMBIAE* AND *AN. STEPHENSI* AT DAWN AND DUSK

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It seems likely that the preferences of female and male mosquitos regarding ambient illumination are intertwined with critical vector behaviors, including endophily, exophily, endophagy and exophagy. Nonetheless, we understand very little about the preferences of malaria vectors regarding ambient intensity of illumination. We have therefore developed and implemented a dual-choice illumination preference assay, a gradient illumination preference assay, and a wavelength preference assay using *Anopheles gambiae* and *An. stephensi*. We have begun with analysis of illumination preferences of both species at subjective dusk and dawn. Dual-choice and gradient assays reveal that female *An. gambiae* females exhibit a high preference index for low illumination in both arenas, whether introduced into the low- or high-illumination zones in these arenas. Male *An. gambiae* also exhibit a preference index favoring low and intermediate illumination, with a magnitude lower than that of female mosquitoes. We will present initial results regarding wavelength preference, spanning the visible spectrum, in *An. gambiae* and *An. stephensi*, and we will analyze the relationship between Zeitgeber time and illumination preferences in both species. These analyses will extend our understanding of illumination preferences in vector mosquitos, and set the stage for analysis of the dependence of these preferences on different rhodopsin family members and on circadian rhythms in vector mosquitos.

1217

THE EFFECT OF TEMPERATURE ON LIFE HISTORY TRAITS OF *CULEX* MOSQUITO POPULATIONS

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Culex mosquitoes,are primary vectors of *West Nile virus* and other pathogens in N. America. Evaluating the life history traits of *Culex* species and populations under variable temperatures is required to define the relationships between temperature, mosquito fitness and vectorial capacity. This is particularly important in light of changing global climates. Temperature is a significant abiotic factor for mosquitoes as it can directly affect development, mortality, morphology, and fecundity. Although the effects of temperature on pathogen proliferation for mosquito-borne agents are generally defined, a detailed understanding of the effects of temperature fluctuations on development of populations of *Culex* mosquitoes is lacking. Additionally,colonized and field populations can differ significantly in genetic and phenotypic diversity, as well as general physiology. Defining these differences is essential for interpreting studies of vector populations, which often rely on highly colonized populations to assess the effects of environmental conditions on vectorial capacity and fitness. We determined the effect of rearing temperatures including 16, 20, 24, 28, and 32^{o}C, on life history traits of colonized and field-derived populations of *Cx. pipiens, Cx. quinquefasciatus*, and *Cx. restuans* mosquitoes. Specifically, we measured temperature-dependent variation in life table characteristics such as development time, immature survival, adult survival, mosquito size, blood-feeding rates, and fecundity both among species and between colonized and field populations. Results demonstrate that all measured traits are significantly affected by temperature in all tested populations, yet also demonstrate that both species and population-specific effects significantly contribute to variation in these traits. In general, temperature and both immature and adult survival are negatively correlated, yet field populations of *Cx. pipiens* and *Cx. quinquefasciatus* survived longer than colony populations, particularly at lower temperatures. In addition, temperature was shown to significantly alter both body size and bloodfeeding rates and optimal temperatures for these traits differed among populations, particularly for *Cx. pipiens*. Taken together; our results demonstrate the significant effects of temperature on life-history traits and identify critical population and species-specific differences.

1218

GENETIC CONTROL OF MOSQUITOES: A WHIRLWIND TOUR OF GLOBAL ACTIVITIES

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Genetic control of mosquitoes has been envisioned, developed, and is now being implemented. Several approaches are currently at some stage of field implementation or are under development and trials are being considered. I will give a very rapid overview of the technologies and the current status of their degree of technology development, potency and field implementation. While the evaluation is obviously subjective, the overview will provide the uninformed with their current status in a nutshell. The overview will cover all genetic approaches as defined by "Dissemination, by mating and inheritance, of factors that reduce pest damage." These include transgenic (RIDL, HEGs, Refractory) and non-transgenic approaches (*Wolbachia* and radiation-sterilized) mosquitoes. "Technology development" indicates my evaluation of how far the technology has matured toward a workable tool. "Potency" will be evaluated in terms of the versatility of the technology, the likelihood that the cost:benefit favors widespread implementation and effectiveness in developing countries and the intrinsic durability of the intervention. "Implementation" is an assessment of whether field activities are actually occurring and in a way that represents a full-blown public health intervention.

1219

RAPID COMPARATIVE EVALUATION OF ANOPHELINE SAMPLING METHODS IN THREE LOCALITIES IN INDONESIA

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Effective sampling techniques are necessary to monitor vector populations and evaluate the effectiveness of vector control interventions. Most methods for monitoring malaria vectors have been developed for trapping anthropophilic, indoor biting African mosquitoes and may not be effective

in areas with diverse, outdoor biting vectors such as those in Indonesia. In this study, eight different collection methods were tested in three different locations in Indonesia representing different malaria transmission zones: a low transmission field site in Purworejo, Central Java; a medium transmission field site in Lampung, Sumatra; and a high transmission area in South Halmahera. Indoor and outdoor human landing catches, CDC light traps, resting pots and boxes, as well as several exposure free tents: the Ifakara Tent Trap model C, large and small human-baited tents were tested for both total number of anophelines per night and species captured. A goat-baited tent was also tested in one of the three locations. Traps were tested in a 3 x 3 Latin-square design and mean anopheline catches per trap night were compared using ANOVA. Samples were morphologically identified and screened by cs ELISA and sporozoite diagnostic PCR and species identifications were molecularly confirmed by sequencing the ribosomal DNA ITS2 region. In Lampung, a total of 2362 anophelines were collected over 16 catch nights, 5 of which were found to be positive for *P. falciparum* and 57 samples positive for *P. vivax*. In Purworejo, 286 total anophelines were collected over 16 catch nights, with 2 samples positive for *P. vivax*. In Halmahera, 63 anophelines were collected over 8 nights, with no *Plasmodium* positives. We conclude that outdoor human landing collection remains the most effective method for collecting malaria vectors in Lampung and Purworejo, and a goat-baited tent (not tested at the other two sites) most effective in Halmahera. Effectiveness of each trap on the 10 different *Anopheles* species captured over the three sites will be presented.

1220

LARVAL SOURCE MANAGEMENT AND MOSQUITO-BORNE PATHOGEN TRANSMISSION

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Mathematical models of malaria transmission have influenced strategic decisions about malaria control since the time of Ronald Ross, when larval source management (LSM) was the dominant mode of malaria prevention. Following the success of early trials using indoor residual spraying (IRS) with DDT, George Macdonald showed that transmission was highly sensitive to adult mosquito mortality rates, which reinforced the prevailing notion at the time that DDT alone was sufficient to eradicate malaria. Recent policy recommendations for vector control did not include LSM, based once again on Macdonald's analysis. Recent empirical evidence demonstrates that, in some circumstances, LSM can be as effective or as cost-effective as IRS or insecticide-treated bed nets. A recent review demonstrated that the mathematical theory underpinning control of terrestrial adult and aquatic immature mosquito populations have not been developed to the same extent, however. No simple theory exists for LSM that is comparable to Macdonald's analysis of adult mortality. Here, we present a simple model describing mosquito population dynamics, update Macdonald's original analysis, and establish basic theory for LSM. One of the key features of our model is that aquatic mosquito populations are distributed among distinct aquatic habitats. With density dependence in these structured aquatic habitats, the effect sizes of LSM respond in a non-linear way with the proportion of treated aquatic habitats. In other words, removing 50% of larval productivity reduces mosquito population density by more than 75%. We discuss the factors, such as spatial distribution of aquatic habitat, variation in productivity among habitats, and mosquito egg-laying behavior, that affect the efficacy of LSM.

1221

PREDICTING *ANOPHELES GAMBIAE* LARVAL HABITAT LOCATIONS IN LOWLAND, WESTERN KENYA

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Malaria risk to humans is heterogeneous at the community scale. The locations of people's houses relative to the locations of larval *Anopheles* habitats have been shown to influence the spatial distribution of malaria infections in certain landscapes. In lowland, western Kenya, *Anopheles gambiae* larval habitats are numerous and widespread, and the relationship between larval habitat distribution and malaria transmission is less clear. Accurately predicting the locations of larval habitats will allow us to test this relationship across a large area. We used geographic information over a 10 by 10 km landscape in Asembo, Kenya to model the locations of *An. gambiae* larval habitats. Thirty-one sample quadrats (500 by 500 m) were exhaustively surveyed, and all potential larval habitats were georeferenced with GPS. A raster data model at 20 m spatial resolution was built around five geographic variables for the study area: elevation, distance to the nearest stream, land cover/land use categories, and two indices of topography related to pooling water (slope:area ratio and locally low index). These variables were tested for their ability to predict habitat locations using logistic regression modeling. Preliminary results suggest the importance of all five variables in predicting the locations of *An. gambiae* larval habitats. Habitats were more likely to be found in areas where the slope:area ratio and locally low index values predicted pooling water. They were also more likely closer to streams and in agricultural land. Future work includes using predicted *An. gambiae* larval habitat locations to explain heterogeneity in adult *An. gambiae* spatial distribution.

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ESTIMATING LONG-TERM *AEDES AEGYPTI* ABUNDANCE IN IQUITOS, PERU USING A NOVEL, SPATIALLY-EXPLICIT SMOOTHING METHOD

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Understanding patterns of spatial and temporal variation in *Aedes aegypti* abundance is crucial for predicting dengue incidence and modeling virus transmission dynamics. Since 1999, over 150,000 entomological surveys were conducted within individual homes throughout urban Iquitos, Peru, using a standardized collection procedure. Based on data from a 1 year period between interventions (n=13,109 surveys), we estimated a mean adult mosquito abundance of 0.577 per house (negative binomial, size = 0.166). Adult mosquito counts were highly over-dispersed and varied greatly across space ($p<0.05$). Accurately estimating adult mosquito abundance over long time periods is complicated, however, by spatiotemporal heterogeneity in the mosquito environment, limitations on mosquito capture efficiency, and the coverage and timing of interventions. To account for variation over the full 13 years of data, we thus develop a non-parametric smoothing approach that creates a single time-series to describe city-wide mosquito abundance as well as a surface that describes spatial differences across the urban landscape. Since we avoid restrictive seasonality assumptions we are able to easily compensate for intervention efforts as well as discern inter-annual variation in the timing of peak mosquito abundance. We are also able to identify, on a small scale, locations that systematically over- or under-produce relative to the rest of

the city. Future work will combine our time-series and surface describing mosquito abundance with spatiotemporally explicit dengue infection data to better understand and predict the patterns of dengue outbreaks.

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MOSQUITOES *(DIPTERA:CULICIDAE)* FROM THE NORTHERN PERUVIAN AMAZON

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An entomological survey was conducted in four villages (Lagunas, Santa Cruz, Saramiriza and Puerto America) 635 km southwest of Iquitos in the northern Peruvian Amazon Basin, to determine mosquito population demographics and predict human risk of exposure to mosquito-borne diseases in this region. Collections were made using CDC light traps placed in extradomiciliary areas (4-20 traps/site, 100 m away from houses) from 1800-0600 hours; protected human bait situated in peridomiciliary areas from 1800-2100 hours; and backpack aspirators inside houses (20 min/house, 50 houses/site) from 0800-1245 hours. A total of 22,513 mosquitoes were identified, belonging to two sub-families, Culicinae and Anophelinae, 12 genera, 14 sub-genera and 48 species. *Culex* and *Mansonia* spp. accounted for 57% and 18%, respectively, of the total mosquitoes collected by all methods combined. CDC light trap captures contained the largest mosquito number and the greatest species diversity (16,947 mosquitoes, 44 species). Human bait (3,888 mosquitoes, 27 species) and backpack aspirators (1,678 mosquitoes, 25 species) captures were smaller. Trap collections reflect typical genera bias: CDC light traps contained 68% *Culex* spp. and 9% *Ochlerotatus* spp., while human bait collections consisted of 75% *Mansonia* spp., and backpack aspirator collections contained 68% *Culex* spp. and 29% *Mansonia* spp. The average hourly collection rates (mosquitoes/hour, m/h) differed between collection methods: 118 m/h for human bait, 88 m/h for CDC light traps, and 15 m/h for backpack aspirators. Identified Anophelinae belong to the subgenera *Anopheles, Nyssorhynchus* and *Stethomyia*. *An. oswaldoi, An. benarrochi and An. mattogrossensis* had the highest density. The Shannon-Weaver diversity index (H) ranged from 0.83 (Santa Cruz) to 1.04 (Lagunas), indicating a potentially high mosquito diversity in the northern Peruvian Amazon, where *Culex* spp. and *Mansonia* spp. are the most abundant. Several of the mosquito species collected in this study are of interest because of their potential role as vectors of arbovirus and parasites in the Peruvian Amazon Basin.

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DEMONSTRATION OF A PUSH-PULL STRATEGY FOR DENGUE VECTOR CONTROL: OBSERVATIONS FROM LOCAL THAI HOUSES

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A pilot demonstration trial for a repellent focused push-pull strategy was conducted at local homes in two Thai villages. The purpose of the trial was to validate findings from experimental hut studies that have been under evaluation for the last 4 years in both Thailand and Peru and to determine the challenges in implementing the strategy under a "real-life" scenario. The goal of the push-pull system is to reduce densities of the dengue mosquito vector, *Aedes aegypti,* inside homes. The strategy has two components: 1) the "push" which involves the placement of a spatial repellent treatment inside homes with the intent to deter the mosquitoes from entering and 2) the "pull" which involves the placement of a mosquito trap outside the home to capture repelled mosquitoes in the peridomestic environment. Combined, the approach would reduce human-vector contact thereby decrease the probability of virus transmission. While experimental hut studies at both research sites have demonstrated efficacy of the push-pull strategy to impact densities of adult mosquitoes entering treated huts as compared to matched controls, and when either tool is used separately, it was necessary to test the strategy at local homes to measure expected changes in efficacy from experimental to natural conditions and define the impact on adult *Ae. aegypti* inside houses where people reside. Such information is vital to provide evidence of effectiveness and applicability in local settings for future strategy development and to establish entomological correlates of impact to drive translational research.

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POTENTIAL FOR DENGUE VIRUS TRANSMISSION IN SOUTH TEXAS

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Semiarid subtropical South Texas is a unique ecoregion in the United States. The geographic and socio-economic conditions of this region may increase the risk of endemic dengue transmission. We conducted an initial study in summer 2010, examining factors that may influence dengue transmission. We studied two potential dengue vectors, *Aedes albopictus* and *Ae. aegypti*, found in the region in the summer of 2010 to identify distribution patterns and relative abundance. We used, oviposition traps to collect eggs from these container breeders and hatched the eggs to identify species. We also surveyed local residents at key field sites to assess behavioral factors influencing dengue transmission. Research questions incorporated knowledge of mosquitoes, frequency of outdoor activity, and awareness of mosquito activity. Our results show that both *Ae. aegypti* and *Ae. albopictus* are present in South Texas, despite a drought that was concurrent to the research study. In addition, human behavior and knowledge varied widely, with a range of survey results showing a population that may be at increased risk due to lack of awareness or increased risk of exposure to the potential disease vectors. Our preliminary study suggests that South Texas may be at increased risk of endemic dengue transmission, and further studies are warranted.

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ANOPHELES ABUNDANCE AND BLOOD FEEDING PREFERENCES IN SOUTH TEXAS

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Anopheles mosquitoes are the primary vectors for the parasitic disease malaria. Endemic malaria has been eradicated from the United States; however there is still a low risk for endemic malaria transmission in Mexico. South Texas may remain at risk for malaria importation due to a shared border with Mexico. To understand the risk of endemic malaria transmission in the Lower Rio Grande, South Texas, we studied the distribution and abundance of the malaria vectors in this region, *An. quadrimaculatus* and *An. pseudopunctipennis*. In addition to studying species composition, abundance, and distribution, we examined blood feeding preferences in order to better assess malaria transmission potential. We conducted a three-month survey of the mosquitoes in the Lower Rio Grande Valley from late June through mid-September using light traps and resting boxes. Four trapping sites along the Rio Grande River from Mission, Texas to Brownsville, Texas were studied. *Anopheles* mosquitoes collected weekly were identified to species. Blood fed *Anopheles* were analyzed with real time PCR to identify blood meal sources with assays designed to distinguish mammal blood meals from other vertebrates. We collected a total of 122 *Anopheles* mosquitoes from three of the four sites, 23 of which had a blood meal prior to capture. Sixteen out of the 23 blood fed *Anopheles* mosquitoes were identified

as feeding on mammalian blood. The presence of *Anopheles* mosquitoes capable of transmitting malaria in the Lower Rio Grande Valley may indicate there is a potential risk of endemic malaria transmission in South Texas.

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BLOOD MEAL IDENTIFICATION USING REAL TIME POLYMERASE CHAIN REACTION

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To control or eradicate vector-borne disease, a detailed understanding of the disease transmission dynamics is required. This requires critical assessment of feeding behaviors, preferences, and strategies of disease vectors, including frequency of feeding on human hosts. Current methods of identifying host feeding preferences include gene sequencing, classical PCR, serological techniques, and ELISA assays. One technique seldom developed is utilizing real-time PCR methods. Real-time PCR techniques allows for the rapid identification of blood meal sources, increased sensitivity to small amounts of blood, and quantification of relative proportions of blood from different hosts. Our new method discriminates between human blood meals, other mammalian blood meals and non-mammalian blood meals. Using comparative genomics of human, other mammals and other vertebrates, regions encoding micro-RNA (miRNA) were identified. MiRNAs are small, less than 120 nucleotides, conserved loci that occur in all organisms. Several miRNAs were screened for their specificity using classical PCR and two were chosen to use for the real-time PCR assay; one identifying humans and one identifying all mammals. The assays were tested for specificity using human, cow, guinea pig, horse, rabbit and dog DNA as positive controls, Asian tiger mosquito, chicken and duck DNA as negative controls. The assays were then optimized on a Cepheid Smart Cycler and Illumina Eco Real Time PCR system using Taqman chemistry. By using a FAM and CalOrange fluorescent probe for each assay, the two can be run as one as a duoplex reaction. Our method provides a rapid, detailed analysis of blood feeding behavior and identifies preferences in host type. Additional assays for avian detection and an internal mosquito control may be developed as well.

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EVALUATION OF POINT MUTATIONS IN THE ATTENUATION OF YELLOW FEVER VIRUS

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Whilst the 17D vaccine has been used extensively for the control of yellow fever virus (YFV), the roles of the specific mutations in its attenuation in both mammals and mosquitoes remain unknown. Previous research has demonstrated the importance of the envelope protein domain III (ED3) in governing the attenuation process and the virulence amongst various flaviviruses. With the cDNA infectious clones of the 17D strain, Asibi strain and chimeric viruses containing the structural region of Asibi strain on the 17D strain backbone, we evaluated the roles of two mutations located in ED3, M299I and T380R, for the infection and dissemination in *Aedes aegypti*. Detection of viruses in the bodies, heads, legs and wings of mosquitoes was based on $TCID_{50}$ titration. Infection was reported based on the detection of viruses in either the whole body or any tissues above. Dissemination was confirmed by the detection of viruses in the heads, wings or legs. The effects of the specific mutations on infection and dissemination in mosquitoes will be discussed.

1229

MODELING THE ECOLOGY OF GENERALIST PATHOGENS IN MULTIPLE MOSQUITO VECTORS

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Models of multi-host, multi-vector mosquito-borne pathogen transmission have been underrepresented considering the issues faced in dealing with pathogens such as sylvatic Dengue virus, Rift Valley Fever and West Nile Virus that are transmitted among several host species by several mosquito vectors. Mathematical models that make different assumptions about the distribution of bites among hosts lead to differing pathogen transmission dynamics. Here we review the multi-host, multi-vector pathogen literature, and present theory unifying and summarizing key aspects of transmission and compare the behavior of major classes of models. We focus on three main areas: tradeoffs between vector preferences and host availability, the role of frequency versus density dependence for vectors and hosts and the roles of host movement and spatial distributions on vector feeding. We mechanistically derive model formulas, present simulations and data informing each of the three areas. Models presented encompass a wide range of ecological scenarios including populations of different species of hosts, meta-populations of a single species separated spatially with or without movement between patches, or heterogeneous biting of a vector on single or multiple host species. Multi-host, multi-vector models encompass pathogens causing significant disease burden. Zooprophylaxis for malaria and the dynamics of pathogens in complex multi-host and multi-vector environments can be further elucidated through the development of theory based on mosquito feeding behavior and ecology and its interactions with host behavior and ecology that lead to patterns of blood feeding. Detailed understanding of transmission cycles of these pathogens could have considerable and direct impact on those living in and around areas with natural zoonotic cycles and for those connected to those populations through human or animal movement.

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DEFORESTATION AND URBANIZATION DRIVE THE INVASION AND TRANSMISSION OF A VECTOR BORNE DISEASE

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Anthropogenic land use has altered much of the planet's surface. This has changed host and vector communities and altered climates. The impact of these changes on the transmission of zoonotic pathogens is difficult to predict because of the numerous interactions among hosts, vectors, and pathogens. We examined detailed aspects of transmission ecology for a vector-borne pathogen, West Nile virus, across a land use gradient from intact forest to highly urbanized cities. We found a strong gradient in transmission potential with viral transmission absent in intact forests and intense yearly transmission in urban areas. This pattern was driven by a combination of changes in vector abundance and species composition, vector feeding patterns, and host community composition, with microclimate playing a smaller role at this spatial scale. These results highlight the key role of changes in the animal community in response to land use change that play an ever increasing role in human health.

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A MATHEMATICAL MODEL FOR *ANOPHELES FARAUTI* ECOLOGY AND MALARIA DYNAMICS IN HALETA VILLAGE, SOLOMON ISLANDS

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Malaria control programs have a variety of available deployable tools, including multiple options for vector control. The potential impact of different vector control tools depends on the local mix of vector species, their ecologies and their feeding behaviors. The EMOD model represents mosquito ecology and feeding dynamics in the presence of interventions in order to study the impact of vector control and other malaria campaign tools upon malaria transmission. The basic model is adapted to study vector ecology and malaria transmission in Haleta village in the Solomon Islands and the local vector *Anopheles farauti*. A new sub-module within the malaria transmission model is created for *An. farauti* ecology and larval habitat, which is primarily a brackish lagoon near the village. Current and historical data for vector feeding behavior are utilized to study the predicted impact of bednets and other vector control tools. Human blood index, vector feeding and resting locations, and timing of feeding are examined for sensitivities upon the impacts of vector control. Given the high rate of outdoor feeding early in the evening and outdoor resting post blood meal, the impact of bednets as a vector control tool is predicted to be limited. The impacts of other vector control tools are simulated to examine possible effects both in combination with and in place of bednets. The local study site, its vector data, and the resulting model outputs demonstrate the importance of understanding vector ecology and behavior in designing locally tailored vector control components of malaria control and elimination campaigns. The VECNet Consortium brings together vector ecologists, tool developers, mathematical modelers, and public health professionals to study these data and their implications.

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MOSQUITO MIDGUT MICROBIOTA PARTICIPATES IN THE CONTROL OF DENGUE VIRUS AND *PLASMODIUM FALCIPARUM* IN HOST VECTOR

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The resident microbiota of insect vectors can impede transmission of human pathogens. Recent studies have highlighted the capacity of endogenous bacteria to decrease viral and parasitic infections in mosquito and tsetse fly vectors by activating their immune responses or directly inhibiting pathogen development. These microbes may prove effective agents for manipulating the vector competence for malaria and other important human pathogens, as well as representing promising sources of anti-pathogen therapeutic natural products. Our purpose was to collect field mosquito and study the natural midgut microbiota allocated in them. We have identified a variety of microbes of the mosquito midgut microflora with potent *in vivo* and *in vitro* activities against the dengue virus and different stages of the malaria parasite *Plasmodium*. Indeed a couple of them show activity against both pathogenic agents. The potential of these microbes for disease control will be discussed.

1233

MOLECULAR AND MORPHOMETRIC ASSESSMENT OF *ANOPHELES ALBITARSIS S.L.* FROM COLOMBIA

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Recent barcode analysis revealed eight species and one lineage within the Albitarsis Complex. Adult females from this complex cannot be differentiated by morphology, but accurate species identification is important for effective and targeted vector control programs. In this study, geometric morphometric (GM) analysis of the wing and *COI* barcode analysis were used to: 1) establish the taxonomic status of *Anopheles albitarsis s.l.* collected in localities of five departments of Colombia and 2) evaluate the potential of GM to separate species of the complex. Preliminary, *a priori* species assignation using DNA barcodes determined the presence of two species, *An. albitarsis* I distributed in north and central Colombia and *An. albitarsis* F found in eastern Colombia. GM analysis of thirteen wing landmarks of type I (venation intersections) of 98 specimens and interspecific wing size comparison showed significant differences between members of both species. *An. albitarsis* I had the smallest wing (average centroid size=3.187 mm±0.019 SE) and *An. albitarsis* F the largest (average centroid size=3.337 mm±0.043 SE). Allometric effect on shape variation was no significant (p>0.05). The interspecific wing shape analysis was non discriminant and non-informative (p=0.144). Subtle differences in wing size could be explained by several environmental variables such as elevation, temperature and food sources in breeding sites. While wing shape was not useful for species delimitation due to morphological overlap, DNA barcoding supported the presence of two species remaining as the method of election for discriminating among these species.

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EXPERIMENTAL STUDIES OF THE DISPERSION OF FEMALES OF TWO *ANOPHELES* SPECIES AROUND INSECTICIDE-TREATED AND UNTREATED BED NETS: IMPLICATIONS FOR ASSESSING THE SIGNIFICANCE OF PHYSICAL DAMAGE TO BED NETS

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Arrays of sticky panels were used to sample released not-previously-blood fed female mosquitoes flying near and landing on sectors of bed nets with and without a person inside. Experiments were done in an environmentally-controlled room with insectary-reared *Anopheles gambiae* G3 or *An. albimanus*, with insecticide-treated (Permanet 2.0®) or untreated bed nets (180x150x130cm) and under four combinations of temperature and humidity (warm-humid, warm-dry, cool-humid, cool-dry). The presence of a person in both treated and untreated bed nets significantly increased total net catch for all temperature-humidity combinations for both mosquito species. For all cases when a host was present, except the case of *An. gambiae* G3 in warm-humid conditions, catches were larger on the sticky panels on the net roof than on panels on the sides and ends. Total catch on treated net panels was somewhat lower than on untreated net panels due to the insecticidal effect but mosquito dispersion patterns did not differ between net types. Results indicate that mosquito pressure varies with location on the bed net depending on mosquito species and ambient conditions. The implications of these findings for situation-appropriate assessment of bed nets that have sustained physical damage in the form of holes, rips and tears is discussed.

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MALARIA VECTOR MOSQUITOES IN NINE KOREAN ARMY-BASE CAMPS NEAR DEMILITARIZED ZONE IN THE REPUBLIC OF KOREA, 2011

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Population density and *vivax* infection rates of *Anopheles* mosquitoes from 27 points (3 points per one base camp; mess hall, guard point and soldier's shelter) in nine Korean army base camps (composed of three General outpost (GOP), three Forward Edge of Battle Area (FEBA) 1 and three FEBA2) near Demilitarized zone (DMZ) in Paju-si and Yeoncheon-gun, Gyeonggi-do, Republic of Korea (ROK) were monitored with black light traps from May to October for supporting basic information for vector control. Members of *Anopheles* mosquitoes were identified to species and were tested for *vivax* infection by Polymerase chain reaction (PCR) with genomic DNA. Of 4,282 mosquitoes, a population density rate of *Anopheles* mosquitoes was over 50% and *An. kleini* (69.5%) was predominant among eight *Anopheles* mosquito species, followed by *An. pullus* (17.3%), *An. belenrae* (4.9%), *An. sineroides* (4.2%), *An. sinensis* (2.7%) and *An. lesteri* (1.9%). No *An. koreicus* and *An. lindesayi* were collected. *An. kleini* exhibited the highest *vivax* infection rates, followed by *An*. *pullus*, *An*. *belenrae*, *An*. *sineroides*, *An*. *sinensis* and *An*. *lesteri*. The *vivax* infection rates of the vector mosquitoes steadily increased from May to October and was the highest in July. Order from highest to lowest mean population density of *Anopheles* mosquitoes was the GOP inside DMZ, the FEBA 1 and FEBA 2 outside DMZ. The mean population density of *Anopheles* mosquitoes in the mess hall in each base camp was higher than that in guard point and soldier's shelter. In our study, *vivax* infected vector mosquitoes in the base camps were collected from May to October and soldiers in the base camps were always exposed to *vivax* malaria during the mosquito season. In the ROK, malaria cases in Korea army base camps near DMZ were over 40% of total malaria cases every year. Malaria management in Korea army is very important to eliminate malaria in the ROK. The effective and accurate vector control should be need from May to October for malaria management in Korea army base camps. Population density and *vivax* infection of vector mosquitoes in Korea army base camps near DMZ should be continuously performed and extend monitoring areas.

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MOSQUITO SPECIES COMPOSITION AND *PLASMODIUM VIVAX* INFECTION RATES IN KOREA ARMY BASE-CAMPS NEAR THE DEMILITARIZED ZONE IN THE REPUBLIC OF KOREA, 2011

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Vivax malaria is a significant military and civilian health threat in the north of the Republic of Korea (ROK). Vector mosquito collections were performed in the Korea two army base-camps in Paju-si near the Demilitarized zone (DMZ) using black light traps in 2011. The DMZ is the northernmost point of the ROK and is located close to the Democratic People's Republic of Korea (DPRK). *Anopheles* spp. were assayed by PCR, to identify the species, and screened for sporozoites of *Plasmodium vivax*. Of 4,354 female *Anopheles* mosquitoes collected, *An. kleini* was the most abundant, followed by *An. pullus*, *An. belenrae*, *An. sinensis*, *An. sineroides*, and *An. lesteri*. *Anopheles kleini*, *An*. *pullus* and *An*. *sineroides* demonstrated the highest population density in June and *An*. *belenrae*, *An*. *lesteri* and *An*. *sinensis* in August. No non-Hyrcanus group species, *An. lindesayi japonicus* and *An. koreicus*, were collected. The value of the total maximum likelihood estimation (TMLE) [estimated number of positive mosquitoes/1,000] for *P. vivax* value of *An*. *lesteri* was the highest, followed by *An*. *sineroides*, *An*. *belenrae*, *An*. *sinensis*, *An*. *pullus* and *An*. *kleini*. The seasonal maximum likelihood estimation (SMLE) values were different depending on *Anopheles* species. *Anopheles belenrae*, *An*. *pullus* and *An*. *sineroides* showed the highest SMLE values in July and *An*. *lesteri* and *An*. *sinensis* exhibited the highest SMLE values in September. *Anopheles kleini* showed the highest SMLE values during August. This is the first report of an *An*. *sineroides* positive for *P. vivax* in the ROK. The results demonstrate new information of *An*. *sineroides* as a potential vector and extend our knowledge of the distribution and a potential role in malaria transmission of vector mosquitoes at Korea army base-camps in areas previously considered to be at a high risk in the ROK for contraction *vivax* malaria.

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SEQUENCE ANALYSIS OF THE INFLUENZA A (H1N1) PDM09 VIRUS HAEMAGGLUTININ (HA) GENE CIRCULATING AMONG INFLUENZA-LIKE ILLNESS (ILI) PATIENTS IN EGYPT

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Influenza viruses and influenza A/H1N1 pdm09 in particular have been associated with a range of clinical presentations from mild to severe and/or fatal disease. Specific genetic mutations in the HA gene of A/H1N1 pdm09 are thought to be associated with virulence and disease outcome. pH1N1 was first identified in Egypt in June 2009, from patients with recent travel history. In this study we analyzed the HA gene of A/H1N1 pdm09 virus circulating among ILI patients in Egypt during the peak season to monitor mutations that could potentially lead to increased virulence. 592 oropharyngeal swabs from ILI patients were collected from November 2009 -January 2010, from eight sentinel sites distributed throughout Egypt, then screened by real-time RT-PCR to detect A/H1N1 pdm09. The virus was isolated and representative isolates were chosen for HA sequencing (Sanger) followed by phylogenetic analysis (MEGA4). Influenza A/H1N1 pdm09 was identified in 27% (n=161) of ILI cases. The HA gene of the 42 representative isolates had 99.1%-99.5% nucleotide identity and 2-8 AA differences from the vaccine strain (A/California/07/2009). All analyzed isolates had the P83S mutation; other mutations including S203T (98%), D222E (71%), P297S (71%) and I321V (86%) were also present. All isolates having the D222E also revealed the P297S mutation; however D222G/N mutations reported to be associated with severe cases were not found. All D222E containing viruses clustered together in the phylogenetic tree. Parallel mutations at certain sites in the majority of the viruses from Egypt may be indicative of active positive selection. Further studies are needed to understand the evolution of the D222E mutation and its effect on receptor binding specificity, antigenicity, transmissibility and virulence of Influenza A/H1N1.

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ETIOLOGY OF FEVER AND ACUTE RESPIRATORY ILLNESS IN NEWBORNS, CHILDREN, AND ADULTS IN A RURAL AREA OF PAKISTAN - A PROSPECTIVE, COMMUNITY BASED SURVEILLANCE STUDY

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Acute respiratory infections (ARI) account for 24% of all under-5 child deaths in Pakistan, translating to an estimated 90,000 deaths annually. There have been no comprehensive studies of the etiology of severe pneumonia in Pakistan since the 1980s. The goal of our study is to determine the etiology of ARI and febrile illness in different age groups in Pakistan, using cutting edge diagnostic modalities including real-time PCR and automated culture methods. This is a longitudinal cohort observational study of newborns, young children, and their adult household members with fortnightly surveillance throughout the duration

of this three year study. A WHO case definition of severe pneumonia is employed, and cases of febrile illness are identified using temperature ≥ 101.3ºF (for newborns) or ≥ 100.4 ºF (for young children and adults). Participants are evaluated by mobile medical teams to obtain appropriate samples and for medical treatment of the observed illnesses. Samples include nasopharyngeal swabs for workup of ARI by PCR identification of bacterial and viral etiologies and blood cultures and malaria ICT for workup of febrile illnesses. In the initial 6 month period of this study, 94cases of severe pneumonia in newborns with or without fever and 8 cases of newborns having fever ≥ 101.3ºF only have been identified. In children 5 to 14 years of age, 5 cases of fever ≥ 100.4 ºF only and 7 cases of ARI without pneumonia have been identified. In adults, there have been 8 cases of fever ≥ 100.4 ºF with influenza-like symptoms and 7 cases of ARI without fever. To date, 35 nasopharyngeal swab samples out of 165 collected from newborns have been analyzed using Luminex assay RVP Fast kit. Of these samples, 16 samples were positive for enterovirus / rhinovirus, 2 were positive for RSV, 5 were positive for PIV type III/IV, and 12 cases were negative for the pathogens tested. Blood cultures were positive in 2 cases, with one case of Campylobacter and E.coli each. Malaria ICT was positive in 2 cases showing combined *Plasmodium falciparum* and *P. vivax* infection. We are currently in the first year of this three year surveillance study. The results of this study will promote establishment of empiric treatment algorithms for ARI and febrile illness in Pakistan.

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POINT-OF-CARE USE OF LED FLUORESCENCE MICROSCOPY COMBINED WITH ULTRASOUND IN THE DIAGNOSIS OF EXTRA-PULMONARY TB: PRELIMINARY RESULTS

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Extrapulmonary TB (EPTB) is difficult to diagnose and early diagnosis and therapy mean-better prognosis and higher survival rates particularly in patients co-infected with HIV. Ultrasound (US) guided fine needle aspiration is crucial in abdominal EPTB in order to obtain samples for direct microscopy and culture, which remains the reference standard. LED Fluorescence Microscopy (LED-FM) is a potential alternative to conventional fluoroscopy in the identification of Acid Fast Bacilli (AFB). To date, this method has been predominantly used in developing countries, but only on sputum. We tested the use of LED-FM in the diagnosis of EPTB in pus samples collected at the patient's bedside using interventional US. Ten patients who were admitted to the Infectious and Tropical Diseases Unit (San Bortolo Hospital, Vicenza, Italy) with suspected EPTB underwent an US-guided sampling of the lesions. Four of them also had HIV infection. Pus was collected from cervical and axillary lymph nodes in 4 patients, in two patients from hilar hepatic lymph nodes, and from mesenteric lymph nodes, abdominal wall abscess and a psoas muscle abscess in the other cases. The samples were obtained by using a Chiba needle (18 G), and were stained and evaluated using LED-FM. Fluorescing microorganisms were seen in all patients (10/10) with auromine/rhodamine stain. Nine cases were confirmed positive at Acid- Fast examination and at culture. In the negative case, an HIV patient with a history of TB, histoplasmosis was the final diagnosis. LED-FM combined with US shows promise as a rapid point-of-care method for the diagnosis of EPTB, particularly in resource-limited settings where EPTB is highly endemic and laboratories are lacking. Studies on large series are needed to evaluate the sensitivity and specificity of this approach.

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PAPER ANALYTICAL DEVICE TO DETECT SUBSTANDARD ANTI-TUBERCULOSIS MEDICATIONS

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The World Health Organization estimates that up to 10% of the world's pharmaceutical trade consists of counterfeit or substandard drugs; it is thought that up to 25% of the drugs consumed in developing countries are substandard. Substandard medicines are products whose composition and ingredients do not meet the correct scientific specifications. Substandard products may occur as a result of negligence, error, insufficient resources or intentional counterfeiting. Counterfeit antimicrobial drugs are a threat to public health and have been linked to increased mortality and morbidity and emergence of drug resistance. I aim to address this problem by developing a Paper Analytical Device (PAD) to identify substandard medications. PADs are inexpensive, user-friendly, transportable, and require no access to power or technology; thus, ideal for use in a developing country. Each PAD is preloaded with a variety of chemical tests to assess the quality of a medicine; a color change indicates the presence or absence of active pharmaceuticals, excipients and known contaminants. Specifically, I am developing a PAD to detect the quality of the first line of anti-tuberculosis drugs: rifampicin, pyrazinamide, isoniazid, and ethambutol. In the last five years, the USP recorded 20 cases of substandard anti-tuberculosis medications in the Cambodia, Philippines, Vietnam, and Peru. Due to the length of treatment for tuberculosis and existing drug resistance, the standard course of treatment utilizes a combination tablet; therefore, the PAD must not only be able to detect pure forms of the drugs, but also drugs in combination. The battery of colorimetric tests on the PAD indicate the presence or absence of a drug both individually and in combination with the other medications. The PAD will also test for known excipients and other active pharmaceuticals that are known to be used in counterfeiting, such as acetaminophen. My work aims to provide clinics in developing countries with the tools to fight against substandard medications and ensure their health.

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PILOT INTERVENTION STUDY OF HOUSEHOLD VENTILATION AND FINE PARTICULATE MATTER CONCENTRATION IN A LOW-INCOME URBAN AREA, DHAKA, BANGLADESH

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Exposure to fine particulate matter ($PM_{2.5}$) is a risk factor for childhood pneumonia in low income settings. Observational studies indicate that household ventilation may be protective against $PM_{2.5}$ exposure. In this pilot study, we tested the effects of behavioral and structural interventions to increase household ventilation on indoor $PM_{2.5}$ in Kamalapur, an urban slum in Dhaka, Bangladesh with infrequent biofuel use. We conducted the pilot between July and August 2011, the hot season when windows and doors are commonly open and fans are on. We recruited good ventilation (window or door in ≥3 walls) and poor ventilation (no window, 1 door) homes using stratified random sampling. We monitored $PM_{2.5}$ for 48 hours inside and outside each home at baseline. We asked participants to increase ventilation behavior by opening windows and doors and turning fans on as much as possible and to keep logs of these behaviors. When physically feasible and permitted, we installed a window in poor ventilation homes. $PM_{2.5}$ monitoring was repeated after behavioral intervention and window installation. We used linear mixed effects models to examine the effect of behavioral and structural interventions

on $PM_{2.5}$ concentrations. We estimated the number of hours that $PM_{2.5}$ concentrations exceeded 50, 100, 250, 500, and 1000 μg/m^3. Compared to 29 poor ventilation homes, 59 good ventilation homes were larger and more likely to be made of concrete; their residents were wealthier, better educated, and more likely to never use biofuel. Among poor ventilation homes, installation of a window was refused by the landlord (n=6, 32%) or resident (n=5, 26%), or we were unable to install the window due to space constraints (n=4, 21%). A window (mean size 0.4m^2) was installed in 10 (34%) homes. After adjusting for covariates, the addition of a new window in poor ventilation households was inversely associated with the number of hours in the 48-hour monitoring period that indoor $PM_{2.5}$ exceeded 250, 500, and 1000μg/m^3 (3.1, 3.9, 3.2 hrs, respectively, $p<0.05$). Compared to baseline there was no significant difference after intervention in the mean number of hours that each door (17.3 v 18.2) and window (18.5 v 19.4 hrs) was open per day. Installation of a window was associated with reduced indoor $PM_{2.5}$, but recommendations to increase ventilation behavior were not. When feasible and acceptable, installation of a small window may help to improve air quality in low income homes.

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PANDEMIC H1N1 INFLUENZA SURVEILLANCE IN HAITI, JULY-DECEMBER 2009

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Influenza is a significant cause of morbidity worldwide, and little is known about influenza in the Caribbean region. In June 2009, following the WHO declaration of influenza A (H1N1) pdm09 (2009 H1N1) pandemic, Haiti established a sentinel surveillance system for influenza. Providers were instructed to collect nasopharyngeal specimens from patients with influenza-like illness (defined as a person with a temperature >38C and cough or sore throat in the absence of other diagnoses) at health facilities in all 10 departments of Haiti. Beginning in July 2009, the Haiti National Public Health Laboratory tested specimens for influenza A and B using real-time RT-PCR and subtyped for pandemic influenza, A/H3 and seasonal A/H1. A subset of samples was sent to CDC-Atlanta for confirmatory PCR and antiviral resistance testing using pyrosequencing and neuraminidase inhibition assays. During July-December 2009, 509 specimens from all 10 departments were collected. 10 were discarded because of lack of identifying information. The majority were from the Ouest department (73%). 197/499 (39.5%) specimens were positive for influenza: 95 (48%) were pandemic 2009 H1N1, 57 (29%) were seasonal influenza A/H3N2 and 45 (23%) were influenza B. The median age of 2009 H1N1 influenza patients was 21.7 years (range: 5 months- 67 years). Two-thirds of 2009 H1N1 were in patients aged 6 months - 35 years. There was no significant difference in the mean ages of patients with 2009 H1N1 and seasonal influenza (22 vs. 25.5, p=0.1). Pandemic 2009 H1N1 activity in Haiti peaked in September. All 11 2009 H1N1, four seasonal and two influenza B specimens tested were susceptible to neuraminidase inhibitors. In Haiti, as in other countries in the region, 2009 H1N1 influenza affected mostly children and younger adults and peaked in early Fall 2009. Additionally, Haiti had co-circulation of influenza subtypes and types.

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EPIDEMIOLOGY OF PNEUMOCOCCAL PNEUMONIA IN ADULTS IN GUATEMALA

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Streptococcus pneumoniae is an important cause of pneumonia worldwide, but little is known about the epidemiology of pneumococcal disease in adults in Latin America due to diagnostic challenges. To describe the epidemiology of pneumococcal pneumonia in adults, we analyzed surveillance data from Santa Rosa and Quetzaltenango in Guatemala during 2008-2011. A case of pneumococcal pneumonia was defined as a hospitalized patient aged ≥18 years with evidence of acute infection (fever, hypothermia, or abnormal leukocyte count or differential), at least one sign or symptom of respiratory disease, and either a rapid urinary antigen test or a blood culture positive for *S. pneumoniae*. We calculated incidence rates for municipalities near the hospitals based on growth-adjusted population denominators from census data. The surveillance system captured 938 adults with evidence of acute infection and a sign/symptom of respiratory disease; 544 (58%) underwent urinary antigen testing and 176 (19%) had blood culture performed. A total of 101 cases of pneumococcal pneumonia were detected; all had positive urinary antigen test and four additionally had *S. pneumoniae* isolated from blood culture. Chest radiograph results were available for 63 pneumococcal pneumonia cases, of which 42 (66%) showed radiologic evidence of pneumonia. The incidence rate of pneumococcal pneumonia was 6.4 per 100 000 persons per year: 3.7 among persons 18-39 years; 5.2 among persons 40-64 years; and 24.7 among persons ≥ 65 years. The case fatality proportion was 8%: 11% (4) among persons 18-39 years; 4% (1) among persons 40-64 years; and 8% (3) among persons ≥ 65 years. Observed rates likely underestimate burden of pneumococcal disease but highlight increased risk among elderly people. These data provide a useful baseline against which to measure the indirect impact of introducing the pneumococcal conjugate vaccine in infants, which has lead to declines in adult pneumococcal disease in other settings.

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HEALTHCARE ASSOCIATED RESPIRATORY SYNCYTIAL VIRUS INFECTIONS AT THREE REFERRAL HOSPITALS IN KENYA, SEPTEMBER 2009 - JULY 2011

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Respiratory Syncytial Virus (RSV) is a major cause of respiratory illness in all ages but occur more often in children < 1 year. RSV presents as a mild infection but can cause severe illness. Children < 2 years with chronic heart or lung disease and Immunocompromized individuals are at high risk of severe RSV infection. RSV is the most common cause of respiratory hospital acquired infection (rHAI) in pediatric wards with up to 45.0% of contacts getting infected. There is limited information on the role of RSV in rHAI in developing countries. This study was conducted to determine the genetic relationship of RSV strains in order to confirm nosocomial transmission from three Kenyan referral hospitals [Kenyatta National

Hospital (KNH), New Nyanza General Hospital (NNPGH) and Mbagathi District Hospital (MDH)]. Nasopharyngeal and oropharyngeal samples were collected from hospitalized patients who developed respiratory illness, 72 hours post admission. A total of 286 samples were screened for the presence of RSV matrix protein using real-time RT-PCR and characterized as RSV-A and -B using a multiplex real-time RT-PCR assay. Of these, 252 had complete demographic data and were included in further analysis. Sixty percent of the patients were from KNH, 22.5% were from NNPGH, and 17.5% were from MDH. RSV was detected in 40 (15.9%) patients with 22 typing as either A (59%), B (31.8%) or AB dual (9.1%) subtype. Seventeen of these positive samples were successfully sequenced. Three RSV- A and 2 RSV-B sequenced samples from KNH were 100% identical in the G ectodomain sequences. One RSV-A specimen from MDH and one RVS-A positive from NNPGH had 100% identity although these two hospitals are approximately 160 miles apart. Three sequences from KNH clustered together with high nucleotide sequence identity as well as high bootstrap support suggesting a common source virus. These results suggest that there were possible multiple introductions of RSV into these hospitals as well as some possible spread of a single strain within one hospital. It is therefore recommended that there should be enhanced infection control measures in hospitals to curb the spread of RSV and other infections in the study areas.

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ETIOLOGY AND IMPACT OF RESPIRATORY INFECTIONS DURING PREGNANCY AND INFANCY ON THE HEALTH OF MOTHERS, FETUSES AND NEWBORNS: A PROSPECTIVE COMMUNITY-BASED SURVEILLANCE STUDY IN PAKISTAN

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In the developing countries, the etiology of common respiratory infections during pregnancy, and the impact of these infections on the health of the mother and fetus are not known. Similarly, the etiology of respiratory illness and fever in newborns, and their impact on the growth and development during the infancy needs to be better understood. This knowledge is important to make appropriate preventive strategies like vaccination during pregnancy and infancy. Our study aims to determine the etiologies of ARI and febrile illness in pregnant women and their newborns in Pakistan, and to determine the long term impact of these infections on the health of mothers, and the growth and development of fetuses and newborns. We are conducting a three-year longitudinal cohort observational study in Bilal Colony, an urban slum of Karachi, Pakistan, where we are following a cohort of 350 pregnant women from the first trimester onwards, and their newborns from birth through the duration of the study period. The study participants are visited every week to check for any ARI episodes or fever. Blood cultures and malaria ICT are obtained for fever > 100.4˚F/38˚C, and nasopharyngeal swabs are obtained for respiratory viral detection in eligible ARI episodes. Anthropometric measures are obtained from all study participants on monthly basis. In the first 6 months of this study so far, the enrollment of pregnant women has been completed, and 123 women have delivered. Out of these, there have been 59 live births, 6 still births and 30 spontaneous abortions. The spontaneous abortions rate is higher than the reported national estimate of abortions, which is about 1 in 7 pregnancies. So far, there have been 3 fever and 3 ARI episodes in pregnant women, and one case of severe pneumonia in the newborn. Continued surveillance of this cohort will enable us to better define the etiologies of ARI and fever in pregnant women and newborns in Pakistan, and the long term impact of these infections on the health of mother, fetus and newborn.

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BIRTH COHORT TO STUDY INFLUENZA INFECTIONS IN INFANTS 0 TO 2 YEARS OLD IN NICARAGUA

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Influenza is a major public health problem worldwide; however, relatively few data exist on influenza in tropical regions, especially in very young children. In September 2011, the Nicaragua Influenza Birth Cohort Study was established in Managua, Nicaragua, to study the burden of influenza in infants and toddlers. The study design allows for the investigation of repeat infections, including immunological characterization, as well as determinants of disease severity. We plan to conduct the study for three years, with 250 infants enrolled each year and followed until 2 years of age, for a total sample size of 750 infants. Each month, approximately 20 newborns, aged 4 weeks or less, are enrolled in the study to maintain the age structure. Infants are contacted on a weekly basis by study personnel, and data on daily symptoms are collected using diary cards. Children are provided with all primary medical care through the study, and 189 variables are collected at each medical visit. All participants presenting with fever or reported fever are tested for influenza by RT-PCR. Yearly blood samples are collected beginning at 6 months of age to enable detection of influenza infections and to evaluate nutritional status. Numerous information and communications technologies are employed to manage study data, track samples, and maintain quality control, including smart phones, tablets, barcodes, global information system (GIS), and scanable forms. We are currently piloting the direct entry of medical visits into tablet computers. To date, acceptance into the study has been high (89.0%), and loss to follow-up/withdrawal has been low (3.9%). As of April 2012, 154 infants have been enrolled, with a median age at enrollment of 17 days. The participants attended 855 medical visits at the study health center. Although the study period to date has not included the influenza season, two laboratory-confirmed influenza cases were detected. A total of 35 participants were transferred to the hospital. Twenty percent of the transfers to the hospital were for respiratory illness; one infant was transferred for bronchitis and 7 for pneumonia. One infant died of pneumonia. In September of 2012, we will complete the first year of the study and perform an interim analysis of results. This study will provide crucial information about the burden of influenza and the incidence and characteristics of sequential influenza infections in infants.

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BIBLIOMETRIC ANALYSIS OF THE IMPACT OF THE 2009 INFLUENZA PANDEMIC ON THE SCIENTIFIC LITERATURE PRODUCTION ON INFLUENZA A

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Influenza A virus causes recurrent pandemics. Concern over avian influenza (H5N1) in recent decades has heightened attention on influenza, spurring greater investment in research and public health preparedness. When the influenza A H1N1 virus (pH1N1) pandemic occurred in 2009, the response was rapid and a large amount of scientific information was published. Bibliometric analysis is a systematic method to evaluate research output through quantity and quality methods. We conducted a bibliometric analysis to measure the impact of the 2009 pH1N1 pandemic on scientific literature production as measured by the immediacy index (II), a measure of how quickly articles in a journal are cited, and impact factor (IF), a measure reflecting the average number of recent citations. A search strategy was built to retrieve original studies on influenza published from

2005-2010 in the PubMed database. II and IF of journals were obtained from the Journal Citation Report. During the study period, 7,046 original studies related to influenza were published in 1,009 journals from 85 countries. 1,980 studies (28%) obtained funding and 145 (2%) were registered as group authorship. The number of original studies, involved journals, funded studies, and group authorships trended upward during the study period, with accentuated growth from 2009. Studies published in 2009 appeared in journals with higher II and IF than previous years, returning to pre-pandemic levels in 2010. Journals that published a high percentage of studies on influenza had greater increases in II (p=0.02) in 2009 and more than twice the number of citations compared to 2008. Increased IF was observed in 2009 for all journals independent of the percentage of influenza studies published (p=0.88). Scientific literature production regarding influenza was increasing even before the 2009 pH1N1 pandemic, partly as a result of concern over avian influenza. However, the pH1N1 pandemic caused a significant increase in influenza research as reflected by increases in the number of studies, participation of journals, funding, and creation of research groups.

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HOUSEHOLD AIR QUALITY IN RURAL PERU: EFFECTIVENESS OF AN IMPROVED COOKING STOVE TO REDUCE EXPOSURE TO INCOMPLETE BIOMASS COMBUSTION

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Half of the world's population depends on biomass fuels to meet domestic energy needs. High levels of emitted pollutants are responsible for substantial morbidity and mortality from lower respiratory infections globally, particularly from household air pollution. The study was embedded in a community-randomised controlled trial carried out in the northern highland, Peru. We determined 48hr household air concentration levels of particulate matter (PM2.5), carbon monoxide (CO) in 93 kitchen environments and personal exposure, seven months after an improved stove (OPTIMA) was installed. We measured simultaneously the personal exposure of mothers and household air in the kitchen using Draeger Pac III datalogger for real-time CO and particle-size-selective Triplex Cyclones for 48hr time-integrated PM2.5 concentrations. Housing characteristics and stove functionality were assessed through questionnaires and participatory observations. Indoor kitchen concentration of PM2.5 and CO did not differ significantly between improved-chimney stoves and traditional stoves. However a post-hoc stratification by functionality levels revealed mean PM2.5 and CO emissions of the well-maintained improved stoves were 28% lower (n=20, PM2.5, 135µg/m3 95%CI 54-216) and 45% lower (n=25, CO, 3.2ppm, 95%CI 1.5-4.9) in the kitchen environment compared to the control stoves (n=34, PM2.5, 188µg/m3, 95%CI 115-261; n=44, CO, 5.8ppm, 95%CI 3.3-8.2), though no statistical significance was observed. Likewise, personal exposures were 44% and 17% lower for PM2.5 (n=23) and CO (n=25). Functionality levels, kitchen volume, type of wood used and the duration the stove was lit were significantly associated with PM2.5 and CO levels in the kitchen, while functionality levels, hours the stove remained lit and the mothers perceiving smoke as a nuisance and contaminant of the kitchen environment were predictors for PM2.5 and CO personal exposure. At the time of sampling, 66% (28/43) of the improved stoves were properly maintained. The results underscore the importance of not only measuring success of household air pollution programmes by the number of installed chimney stoves, but rather by assessing quality of the installation and maintenance of the devices, adoption and continuous use over time. Improved stove programmes should consider both, determinants for sustainability- and functionality in designing future sustainable interventions.

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INFLUENZA-ASSOCIATED SEVERE PNEUMONIA RATES IN CHILDREN YOUNGER THAN FIVE YEARS IN EL SALVADOR DURING 2008-2010 SEASONS

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Disease burden attributable to influenza is poorly understood in tropical low-income countries. To guide health policy, we estimated rates of severe pneumonia associated with influenza among children aged < 5 years in El Salvador. Each week, physicians identified and collected data on all children aged <5 years-old hospitalized with severe pneumonia. Nasal and oropharyngeal swabs were collected for influenza testing on a convenience sample of case-patients. We conducted a health utilization survey in the hospital catchment area to determine the proportion of residents who sought care at the sentinel-site. We estimated rates of severe pneumonia using surveillance and census data adjusting for health service utilization. Influenza strains were characterized by CDC. Influenza strains were compared with northern hemisphere and southern hemisphere vaccine formulations. Physicians identified 2,554 severe pneumonia of which 6% (37/608) of those tested were positive for influenza. The rate of severe pneumonia among children <5 years-old was 1.5/1,000 person-years (py) during 2008, 7.6/1,000 py during 2009 and 0.6/1,000 py, during 2010. Both northern and southern hemisphere vaccine formulations matched isolated influenza virus strains during 2008 and 2010. Influenza hospitalizations were common in El Salvador, but incidence varied substantially among influenza seasons. Both northern and southern hemisphere influenza vaccines were well-matched to circulating strains. Consideration of expansion of influenza vaccination programs may be warranted.

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THE SOCIAL AND ECONOMIC COST OF UNDERGOING TREATMENT AS A TB PATIENT IN GHANA

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Although TB treatment in Ghana is free anecdotal reports indicate that economic and social costs affect their ability to seek appropriate health care. TB programs therefore need to ensure that the economically and socially disadvantaged groups do not face barriers that keep them from seeking treatment. The Poverty Sub-Working Group of the Stop TB Partnership developed a tool which can assist TB programs estimate the costs of TB patients before and during diagnosis and during treatment. This tool was implemented by the Dodowa Health Research Centre and the National TB control Program in Ghana to estimate the costs of TB patients before and during diagnosis and during treatment. It was implemented in all districts in the Upper East region (a deprived region) and two districts in the Eastern region (a better endowed region) and

was administered to new TB patients, older than 15 years, who had received at least one month of TB treatment and given consent. Data was collected through informal discussions with TB coordinators and facility heads, desk top review of patient records and intervies with TB patients to assess direct costs prior to being diagnosed and direct and indirect cost of current treatment. Of the 159 patients interviewed, 64% were in the three lower socio economic quintiles with monthly income less than US$ 42.55. Health system delay was estimated at 1.4 weeks with males taking longer to seek care than females. DOTS patients paid a mean total direct cost of US$ 0.50 for each visit and spent of 58 minutes per DOTS visit. The mean number of days spent in the hospital was 22.7 days and direct cost of hospitalization was US$ 48.32. Whilst 48.3% of the patients borrowed, 37.7% sold assets to cope with paying for their ill health. Reduction of monthly household and patients income due to TB was 44.5% and 82.6% respectively. Sixty-one percent of the TB patients lost their jobs, 11% got separated from their spouse/family and stopped attending public functions. Through this study, the NTP has identified constraints faced by TB patients and their families that have an effect on case finding and treatment adherence. We recommend that TB patients and their families should benefit from social protection packages that will ease the financial burden. Employers should not hesitate to take back workers who have been diagnosed and treated with TB.

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CLINICAL OUTCOMES ASSOCIATED WITH ROUTINE USE OF INTERFERON-Γ RELEASE ASSAYS IN A CENTRAL LONDON TB SERVICE

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Interferon-γ release assay (IGRA) has been shown to have higher specificity than tuberculin skin testing (TST) for screening for latent TB and is recommended by the National Institute for Clinical Excellence (NICE). The aim of this study is to review the indications for and analyse the results of IGRA testing in our central London BCG vaccine positive population. We analysed routinely collected clinical and demographic data on patients referred for IGRA testing to a TB service at a large London teaching hospital from September 2007 to January 2012. Reasons for referral for screening included contacts of active TB, pre-monoclonal antibody therapy, recent migrants and occupational health. Quantiferon Gold InTube was used. We used the London TB registrar to identify patients that were diagnosed with active TB either by bacteriological or clinical evidence from November 2007 to February 2012. We determined the sensitivity and specificity of IGRA for diagnosing active TB and screening for latent TB. We used univariate linear regression to assess the incremental impact of IGRA result on having a diagnosis of active TB. 961 IGRAs were performed on 917 patients. 51% were male with a median age of 30 years (IQR 19-40). There were 46 (4.8%) indeterminate, 703 (73.2%) negative and 212 (22%) positive results. Indeterminate results were more prevalent amongst immunosuppressed than immunocompetent patients 66.7% (24/36) vs. 33.3% (12/36). 46 cases of active tuberculosis were identified from the London tuberculosis register, 15 of which had a negative IGRA result. The sensitivity and specificity of IGRA for diagnosing active TB were 67% and 79% respectively. We found a direct correlation between a positive IGRA test result and active TB diagnosis (p 0.00 coefficient 1.343). The sensitivity and specificity of IGRA for latent TB screening were 55% and 89% respectively. We found an overrepresentation of indeterminate results amongst immunosuppressed patients. IGRA was used in addition or other conventional diagnostic modalities for the diagnosis of active TB, which is outside the scope of the NICE guidelines. Although the use of IGRA for this purpose is approved by the US Food and Drug Administration, caution should be exercised due to its low sensitivity for diagnosing active TB. However the usefulness of IGRA in screening for latent TB was conferred by its high specificity in our setting.

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EVALUATION OF HOUSEHOLD LEVEL INTERVENTIONS DURING A LARGE, URBAN TYPHOID FEVER OUTBREAK - HARARE, ZIMBABWE 2011-2012

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Between October 2011 and March 2012, ~2,750 suspected cases of typhoid fever in two high-density suburbs of Harare (Dzivaresekwa and Kuwadzana) were reported to the Harare City Health Department (HCHD). To prevent outbreak spread, HCHD and non-governmental organizations conducted door-to-door health and hygiene education and distributed point-of-use water treatment (PoUWT) products beginning in October 2011. To evaluate the effectiveness of these interventions, we conducted cross-sectional household surveys in these two affected suburbs in March 2012, including free chlorine residual (FCR) testing in stored drinking water. Reported intervention coverage was high, with 351 (77%) of 458 randomly selected households having received both typhoid fever prevention information and at least one PoUWT product. Of 368 households that received at least one of the three types of chlorine tablets distributed, 326 (89%) reported ever using them, 160 (43%) reported using them daily, and 98 (27%) had stored water that was treated and had FCR ≥0.2mg/L when tested. Only 169 (55%) of 310 household respondents who had chlorine tablets on the day of the survey knew the correct volume of water to treat with their tablets. In univariate analysis, respondents who had higher income, were older, had received PoUWT products or typhoid fever prevention information, and who reported household water treatment before the outbreak were more likely, and respondents who reported boreholes as the primary source of drinking water were less likely, to report water treatment during the outbreak or on the day of the survey, and to have treated stored water with FCR ≥0.2mg/L ($p < 0.05$). The findings highlight: 1) relatively low uptake of PoUWT after free distribution (consistent with other research); 2) the need to improve coordination of NGO response activities through consistent PoUWT product choices and communication about product use; and, 3) the need to emphasize treating drinking water from all sources daily to control and prevent typhoid fever and other waterborne disease outbreaks.

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EFFECTS OF ENVIRONMENT ON HUMAN CYTOKINE RESPONSES: ROLE OF URBAN VERSUS RURAL RESIDENCE

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Environment may have a key role in the development of the immune system in childhood and may explain the low prevalence of allergic and autoimmune diseases in the rural tropics. To investigate the effects of urban versus rural residence on the immune response, we recruited 440 school children living in either in rural communities in the Province of Esmeraldas or in the city of Esmeraldas. We collected data on

environmental exposures by questionnaire and on intestinal parasites by examination of stool samples. Whole blood was stimulated with mitogen, parasite antigen and aeroallergens. IFN-γ, IL-5, IL-10, IL-13, and IL-17 were measured in supernatants. Overall, urban children had mothers with greater educational levels, were more likely to have access to piped water (urban 98.7 % vs. rural 1.9%) and were more likely to use latrines or water closets for defecation (urban 94.8% vs. rural 54.7%). Rural children were more likely to be infected with geohelminths (urban 73.5% vs. rural 20.9%). The frequencies of children with .. DDDdetectable levels of cytokines were similar in urban and rural samples except for IL-10 that was significantly more frequent in the urban population when measured as spontaneous production (adjusted OR 2.56, 95% CI 1.05-6.24) and after stimulation with *Ascaris* (adj. OR 2.5, 95% CI 1.09-5.79) and house dust mite (adj. 2.24, 95% CI 1.07-4.70) antigens. Our data do not provide support for a major role for place of residence or geohelminth infections as a major determinant of the cytokine response in childhood. Surprisingly, urban residence that might be considered to be a more hygienic environment, was associated with more frequent production of the immune regulatory cytokine IL-10.

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PROGRESS ON MDG 7.C IN THE MILLENNIUM VILLAGES AFTER THREE YEARS: IMPROVED HOUSEHOLD WATER AND SANITATION

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Benefits of improving water and sanitation can influence health, educational, employment, economic and social domains. Since 1990, there have been significant global gains in access to improved water, and slower gains in sanitation. Despite commendable improvements, global progress has been uneven, with sub-Saharan Africa, and rural areas in particular, carrying a disproportionate burden of poor access. This mixed-methods implementation study assesses progress towards MDG 7.C across nine sites in rural sub-Saharan Africa in the first three years of the Millennium Villages Project (MVP), a 10-year multi-sector development project. Details of costs, variability between and within sites, challenges and lessons learned are explored in the study. Across nine MVP sites, the proportion of households not using an improved household water source reduced from 87.3% at baseline (2006/07) to 22.7% at year 3 (2009/10) (64.6% percentage-point change, 95% CI = 60.7-68.6%, p-value <0.0001). This represents a 74% reduction in the proportion of population without access to improved water, and exceeds the MDG target for water at a local level, as well as meeting the sub-Saharan African regional target of less than 25% of the population without coverage by 2015. The proportion of the population reporting not using an improved sanitation facility reduced from 98.1% at baseline to 71.4% at year 3 (26.7% percentage point change, 95% CI 24.6%-29.0%, p-value <0.0001). This represents a 27% reduction in the proportion of the population without access to improved sanitation facilities. Although not yet meeting the MDG for sanitation, if the same rate of change were to continue from today to 2015, sanitation would also be on track to meet local and regional MDG targets. These data provide promising evidence suggesting that with MDG-focused interventions, significant gains can be made in household access to improved water and sanitation facilities in a rural sub-Saharan African setting.

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FARM WORKER HYGIENE AND HAND SANITATION IN MEXICO ASSOCIATED WITH CONTAMINATION OF FRESH PRODUCE

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Produce-related foodborne disease outbreaks lead to economic losses, illness, and death, making produce-contamination an important public health concern. In this study we investigated the pathways of produce contamination on Mexican farms, and the sanitation and hygiene practices that may contribute to contamination. We quantified the fecal contamination on produce, hands and environmental samples from 11 farms in Mexico. Produce (cantaloupe, jalapeño, tomato) rinses (161), were collected along with matched irrigation water (89), soil (55), and farm worker hand rinses (106). From these samples, fecal indicators (*E. coli*, Enterococcus, coliforms) and human pathogens (Salmonella, *E. coli* O157:H7) were enumerated. Multivariate regression modeling was used to identify associations. Data were also collected on farm sanitation and worker hygiene through surveys and interviews. Produce was frequently contaminated, 29%-100% of samples were positive for indicators, and the mean concentration of indicators ranged from 10^2-10^6 cfu/fruit. The presence and levels of indicators on soil and water were not significantly associated with those on produce samples. Microbial indicators on hands were significantly higher ($p < 0.05$) than in water or soil. Presence of *E. coli* was significantly associated between hands and produce (OR 7.9, 95%CI [3.3-19.1]). The levels of *E. coli, Enterococcus*, and coliforms (rho=0.4, 0.5, 0.6) were significantly and highly correlated between hands and produce. These data show that hands are a potential source of produce contamination. Hand contamination is likely due to lack of sanitation/hygiene facilities. There were five toilets total available on all 11 farms. Only three had handwashing stations nearby. Evidence of open defecation was observed on two farms. Improved hygiene facilities and sanitation policies on farms could reduce microbial contamination of produce and improve working conditions for employees. Future study aims include the development of training modules on sanitation/hygiene behaviors tailored to farm managers and workers.

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WASTEWATER IRRIGATED FARMS AS A COMMON DENOMINATOR FOR MALARIA AND DIARRHEAL DISEASE TRANSMISSION IN URBAN GHANA

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Worldwide, wastewater use in urban agriculture is increasing at a rapid pace due to urbanization and its accompanying stress on limited freshwater resources. The economic and food security benefits associated with wastewater use in urban agriculture are enormous, but so also are the public health burden the practice exerts on the urban population. Wastewater use in urban agriculture is identified as a major risk factor for diarrhoeal disease, particularly among urban farmers and their family members and consumers of wastewater farm products. Malaria mosquito vectors also breed in wastewater farms, thus exposing nearby urban populations to increased malaria risk. In addition, agro-chemicals used in wastewater farms may contribute to development of insecticide resistance in malaria vectors, further exacerbating the malaria problem. So far, little is known of the contribution of wastewater farms to the combined risk of diarrhoea and malaria. Understanding this contribution would be important for developing integrated interventions. The main

aim of this study is to assess the contribution of urban wastewater farms as a common denominator for the transmission of malaria and diarrhoeal disease in urban Ghana. The study is being conducted in Kumasi, the second largest city in Ghana, where more than 12000 hectares of urban vegetable farms are irrigated with wastewater. The outcome of the study will lead to the development of integrated interventions for mitigating malaria and diarrhoeal disease transmission associated with wastewater irrigation.

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ASSOCIATION BETWEEN EFFICIENTLY COLLECTED MEASURES AND OBSERVED MEASURES OF HANDWASHING BEHAVIOR IN THE IMPACT EVALUATION OF THE GLOBAL SCALING UP HANDWASHING PROJECT IN VIETNAM

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Handwashing reduces diarrhea incidence but is difficult to measure. Structured observation, a direct approach to measuring handwashing behavior, is costly and time-consuming. Self-report, observation of handwashing materials, and visual inspection of hand cleanliness are inexpensive and timesaving and thus efficient measures. We sought to assess whether these measures are associated with handwashing behavior measured by structured observation. With data from controls in the Impact Evaluation of the Global Scaling Up Handwashing Project in Vietnam, we used multilevel logistic regression to calculate wealth-adjusted odds ratios for associations between efficient measures of handwashing and observed handwashing behavior among caregivers, while accounting for multiple events per caregiver. We examined handwashing events overall and stratified by event type. We examined 1379 events overall; 289 fecal contact events (24% accompanied by handwashing with soap) and 569 food related events (6% accompanied by handwashing with soap). Soap and water at the handwashing places used post defecation (OR= 3.96, 95%CI: 1.61-9.53), and before food preparation (OR= 2.34, 95%CI: 1.17-4.68) as well as a rating of ≥7 on a hand cleanliness scale of 1 to 9 (OR= 3.01, 95%CI: 1.75-5.71) were significantly associated with observed handwashing with soap overall. Self-report of handwashing with soap after fecal contact (OR= 4.29, 95%CI: 1.68-11.01), observation of soap and water at the handwashing place used post defecation (OR = 8.21, 95%CI: 1.12-60.24), and hand cleanliness index ≥7 (OR = 3.48, 95%CI: 1.67 - 7.31) were all significantly associated with observed handwashing with soap after fecal contact events. Self-report of handwashing with soap before feeding a child was the only efficient measure associated with observed handwashing with soap before food related events (OR = 4.00, 95%CI: 1.14-14.02). In Vietnam, self-report of handwashing, presence of handwashing materials, and examination of hand cleanliness were associated with observed handwashing with soap overall, and handwashing after fecal contact. Where structured observation is infeasible due to cost, efficient measures of handwashing may be appropriate for measuring handwashing behavior. More importantly, promotion of handwashing with soap is required in Vietnam to improve hand hygiene at critical times relevant to pathogen transmission.

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FAECAL CONTAMINATION OF FOOD, WATER, HANDS AND KITCHEN UTENSILS AT HOUSEHOLD LEVEL IN RURAL AREAS OF PERU

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The aim of this study was to evaluate sources of contamination of child's food and drinking water from rural households in the highlands of Peru. Samples from child meals, drinking water, kitchen utensils, and caregiver and child hands, were analysed for total coliforms and *Escherichia coli* counts using Petrifilm™EC. Thermotolerant coliforms were measured in water using DelAgua® test kit. Diarrhoeagenic *E. coli* were searched by Polymerase Chain Reaction methods (PCR). Thermotolerant coliforms were found on 48% of water samples. *E. coli* was found in 23% of hands, 16% of utensils and 4% of meals. Kitchen cloths were the most frequently contaminated with total coliforms (89%) and *E. coli* (42%). Diarrhoeagenic *E. coli* was found in 33% of water, 27% of meals and 23% of kitchen utensils. There is a need to develop effective hygiene interventions focused to specific kitchen utensils and handwashing, to reduce the contamination of food, water and kitchen's environment in these rural settings.

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WATER- AND SANITATION-RELATED RISK FACTORS FOR SOIL-TRANSMITTED HELMINTH INFECTION IN URBAN SCHOOL- AND PRESCHOOL-AGED CHILDREN IN KIBERA, NAIROBI

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Individuals living in urban slums have limited access to city services, including water and sanitation (WS). Some evidence suggests that WS factors affect risk for soil-transmitted helminth (STH) infections, which disproportionally affect school-aged (SAC) and preschool-aged children (PSAC), but further characterization of WS factors and their impact in slum settings is needed to identify intervention points. Households (n=1,192) containing an index PSAC (6-59 months) or SAC (5-14 years) were randomly selected from those enrolled in CDC's International Emerging Infections Program, a population-based surveillance system in the urban slum of Kibera in Nairobi, Kenya. Data collection included a household-level questionnaire and environmental assessment for WS risk factors and index child stool specimens tested for STH ova by the Kato-Katz method. Stools of siblings living with index SACs were also tested. Household WS factors were classified by the WHO/UNICEF WS service level ladders categorizing resources in groups such as improved, unimproved, and shared, and tested for associations with STH infection. Among 130 households with sufficient data for interim analysis, household STH prevalence (≥1 child in the household positive for any STH) was 36.2%. Of all households, 3.1% reported piped water on premises and 96.9% another improved drinking water source; 62.2% (79/127) of these sources were unofficial connections into nearby municipal pipes. Ever having

difficulty meeting household daily water needs was reported by 76.2% of households, most often due to financial barriers (69.8%). Overall, 2.3% of household sanitation facilities were improved, 87.7% shared, and 6.2% unimproved; 2.3% of households practiced open defecation. Sewage observed in the participant's yard was associated with household STH infection (Fisher's Exact Test, p=0.03). Other associations emerging with ongoing data collection will be discussed. The Kibera population faces gaps in water availability and sanitation quality; STH control here and in similar settings requires an integrated approach.

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A QUALITATIVE EVALUATION OF HAND DRYING PRACTICES AMONG KENYANS

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Recommended disease prevention behaviors of hand washing, hygienic hand drying, and covering one's mouth and nose in a hygienic manner when coughing and sneezing appear to be simple behaviors but continue to be a challenge to promote successfully and sustain globally. We conducted a formative inquiry to better understand current hand drying behaviors associated with activities of daily living, and mouth and nose covering practices, among Kenyans. We conducted 7 focus group discussions (N=45); 30 in-depth interviews; 10 structured household observations; and 75 structured public observations in rural and urban Kenya communities. Using a grounded theory approach, we transcribed, coded, and analyzed the narrative data. Hand drying with a towel is not a common practice. Most women dry their hands on their leso (rectangular cloth wrapped around the waist) or their clothes when cooking, eating, or cleaning a young child. When men dry their hands, they use their trousers or a handkerchief. Children rarely dry their hands but, if they do, they usually wipe them on their clothes. People drew distinctions between hand drying after sporadic sneezing and blowing their nose during a cold. Many people sneeze into their hands and wipe them on their clothes. Men and women tended to use a handkerchief when they had a cold. Drying hands on dirty clothes and lesos can compromise the benefits of handwashing. Coughing and sneezing into an open hand can help spread disease. Health education and promotion materials and messages should stress hygienic hand drying practices such as using a clean towel or cloth, or air-drying. Messages should be particularly tailored to household activities and should emphasize the potential role of dirty towels or clothes as a vector of disease. The importance of sneezing or coughing into the upper arm or a handkerchief should also be emphasized more prominently. Research into barriers to adopting these simple practices is needed.

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POST-IMPLEMENTATION EFFECTIVENESS OF FOUR HOUSEHOLD WATER TREATMENT TECHNOLOGIES IN TYPICAL-USE CONDITIONS IN RURAL KENYA

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Household water treatment technologies are used by about 18 million of the 884 million people without adequate access to safe water. The efficacy of household water treatment technologies has been demonstrated in controlled situations such as laboratory and field trials. However some authors query the sustainability of the efficacy of HWT technologies under real-life situations after the field trials have ended. In view of the dependence of rural communities on highly polluted surface water sources; the sectoral promotion of household water treatment (HWT) systems and the lack of data on their post-intervention effectiveness, it is necessary to evaluate the effectiveness of household water treatment technologies within a real-life context. This study was carried out one and two years after the two implementing organisations had ended their intervention. It examined the microbial efficacy of Aquatab, PUR, Waterguard and ceramic filters by carrying out three unannounced visits between March and April 2010 to each of the 37 HWT user households in five villages in the Nyanza province of Western Kenya. A total of 247 samples were collected from study households' collection and storage containers in order to determine the efficacy of the technologies on water from the 11 unimproved and improved water sources used by the study households. The findings indicate that the four HWTS technologies assessed are able to improve microbial quality of the improved and unimproved water sources. However, based on the observation of inconsistent performance, none of the technologies achieved the minimum expected reduction value or can be classified as a highly protective or protective technology. It is recommended that the drinking water supply and sanitation sector should address the reasons for their reduced effectiveness in the typical-use conditions when compared to laboratory efficacy. These include incorrect usage and inappropriate selection of HWT options for water source characteristics.

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VALIDATION OF AN INDEX OF PROXY MEASURES AND SELF-REPORTED HANDWASHING BEHAVIOR IN DHAKA, BANGLADESH

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Handwashing with soap reduces diarrhea, a leading cause of death in young children. Structured observation permits direct measurement of handwashing, but is inefficient and costly. Measures of handwashing behavior can be efficiently collected by rapid observation and self-report, but in isolation, they are not good indicators of behavior. Using data from primary caregivers in a case-control study of pneumonia risk factors in Dhaka, Bangladesh, we sought to develop an index of efficiently collected handwashing measures, and then test the validity of the index by comparing it to handwashing behavior measured by structured observation. We used principal component analysis, a data reduction technique, to generate a handwashing behavior index score for each caregiver based on handwashing demonstration, rapid observation, and self-reported handwashing behavior. We assigned caregivers to handwashing index quintiles and used logistic regression to compare quintiles to observed handwashing with soap after fecal contact in a 5 hour structured observation, accounting for repeated events. We observed 1,958 fecal contact events, of which 773 (39%) were followed by handwashing with soap. Duration of lathering during a handwashing demonstration, use of soap during a handwashing demonstration, presence of soap at a handwashing station, and self-reported frequency of handwashing accounted for 52% of the variance in the handwashing index score. When compared to those in the lowest index quintile of handwashing scores, each quintile except the third was associated with an increased odds of observed handwashing with soap [2nd quintile OR=1.41, p=0.03, 3rd quintile OR= 0.96, p= 0.81, 4th quintile OR=1.34, p=0.05, 5th quintile OR=1.30 p=0.08]; however, there was no significant linear trend (p trend=0.11). The use of principal component analysis to develop a handwashing behavior index did not identify progressive increases in observed handwashing behavior. Alternatives for index construction, such as summing of items, factor analysis, and prediction modeling, may be considered and evaluated against structured observation and health outcomes data. The construction and validation of indices represents only one approach to addressing the pressing need for low-cost, efficient, and reliable handwashing measures for use in modestly funded studies.

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EVALUATION OF THE MICROBIOLOGIC SAFETY OF STORED RAINWATER AS AN IMPROVED DRINKING WATER SOURCE FOR COMMUNITIES IN KHON KAEN, THAILAND

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Rainwater (RW) is considered an improved source of drinking water (DW) by the WHO and UN agencies tracking progress towards achieving the safe water access target of the Millennium Development Goals. There are, however, a paucity of data on the microbial quality of RW, making uncertain its safety as a DW source. The objective of this work was to evaluate the microbial quality of stored RW collected in a rural village in Thailand using the WHO DW quality guideline value of <1 *E. coli*/100mL as the basis for safety. In 2011, 59 households in Khon Kaen province, all of which used RW as their primary source of DW, were visited twice, once during dry season and once during rainy season. Observational data related to the physical/sanitary conditions of RW harvesting systems (RWHS) were collected during visits. Sampled containers included each household's main RW collection tank and the refillable container used to store RW for daily consumption. Samples were assayed for *E. coli* by the Colisure Quantitray 2000 method and results were scored as present if *E. coli* was ≥1/100mL. Of all samples processed (collection tank, refillable container), 39% and 82% of households had *E. coli* present in at least one container during the dry and wet seasons, respectively. *E. coli* was present in 21% and 66% of RW collection tanks during the dry and wet seasons, respectively. Initial analysis suggests that no single factor related to RWHS setup (roof, pipe, or tank material) had a statistically significant impact on the presence of *E. coli* in RW collection tanks. These results suggest that stored RW microbiologic quality may be highly seasonal, may not always meet WHO guidelines for safe DW, and that deterioration of the microbiologic quality of stored RW is likely due to a combination of collection and use practices. These results document that the UN Joint Monitoring Program's use of access to improved water supplies as an indicator of progress towards the MDG safe water target results in overestimation because improved sources, like harvested RW, may be microbiologically unsafe.

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REDUCTIONS IN DIARRHEA AND CLINIC VISITS FOR DIARRHEA AMONG CHILDREN UNDER THE AGE OF FIVE ASSOCIATED WITH A SCHOOL-BASED WATER SUPPLY, SANITATION AND HYGIENE INTERVENTION IN WESTERN KENYA: A CLUSTER-RANDOMIZED TRIAL

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While many studies have documented reductions in diarrhea incidence in children under five associated with improvements in water, sanitation, and hygiene (WASH) in the domestic environment, the effect of institution-based interventions are not well understood. We conducted a cluster-randomized trial of school-based WASH interventions in 185 public primary schools in western Kenya. Enrolled schools with a nearby water source (<1KM) were randomized into a handwashing promotion and water treatment intervention [HP&WT], HP&WT plus an additional sanitation component [San + HP&WT], or a control group. Schools without a nearby water source were randomized to receive a water supply intervention in addition to the San + HP&WT intervention components or to a control group. Interviews were conducted in a systematic selection of households in the catchment areas of all enrolled schools. Parent reported diarrhea episodes in the past week and clinic visits for diarrhea or vomiting in the past two weeks were recorded for all children under the age of five. Data were collected at baseline (March-April 2007) (n = 4,549) and two years after the start of the interventions (n = 4,392). There was a non-significant 33% reduction in the relative risk (RR) of diarrhea and 51% reduction in the RR of clinic visits among children under five living in the catchment areas of schools receiving water supply improvements compared to control areas (p = 0.185 and 0.075, respectively). Restricting analysis to those children under five living with at least one child attending a school enrolled in the trial increased both the magnitude and significance of this effect (RR diarrhea: 0.53, p= 0.049; RR clinic visits: 0.39, p= 0.03). The HP&WT and San + HP&WT interventions showed no effect on either outcome in both unrestricted and restricted analyses. Our findings suggest that an integrated school WASH intervention that includes the provision of improved water supply at the school can result in substantial reductions in child morbidity even among those children too young to attend school.

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EFFICACY OF DISINFECTANTS ON VIABILITY OF FOODBORNE BACTERIA AND ON *CRYPTOSPORIDIUM* AND *CYCLOSPORA*

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E. coli STEC, *Salmonella*, *Cryptosporidium*, *Cyclospora*, and microsporidia are causative agents of diarrheal illness worldwide. Most of the outbreaks associated with contaminated foods have implicated fruits and vegetables that have been consumed raw. The objective of this study was to determine the recovery efficiencies to improve the methodology detection for foodborne parasites to be used in surveillance studies, to determine efficacy of sanitizers used in the food industry on the survival of foodborne bacteria and parasites, and examine cross contamination during produce harvesting. Five *Escherichia coli* O157:H7, five *Salmonella* spp, and one isolate of each *Cyclospora cayetanensis*, *Cryptosporidium parvum*, and *Encephalitozoon intestinalis* were used for these experiments. Four different wash solutions were examined for recovery of 1000 oocysts of *Cryptosporidium* and *Cyclospora* from 25 gr basil leaves: water, 0.1M phosphate buffer, Glycine, and 3%levulinic acid/SDS. Phosphate buffer worked best. Detection of both organisms was done using nested PCR. Experimentally inoculated basil and bean sprouts were blanched (65, 88, and 100C) and determined if this process kills contaminants. The bacterial contaminants were reduced but not eliminated. The times and temperatures effective for pathogen destruction affected the fresh appearance of vegetables. Freezing did not inactivate bacterial pathogens but *Cryptosporidium* and microsporidia were very sensitive to extreme temperatures. Cross-contamination can occur when contaminated water or contaminated coring tools are used during lettuce harvesting. Reduction of contaminants in lettuce and coring tools was achieved when coring tools were rinsed with diluted chlorine (commonly used in agriculture) and more yet when rinsed with 3% LA/SDS. Sequential contamination of lettuce heads with microsporidia was also observed. Because parasites are highly resistant to chemical disinfectants, it is important to prevent crop contamination during harvesting.

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CHARACTERIZATION OF THE ETIOLOGY AND EPIDEMIOLOGY OF CENTRAL NERVOUS SYSTEM INFECTIONS IN GEORGIA

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Central nervous system (CNS) infections are caused by a large spectrum of viruses and bacteria, and associated with severe and disabling sequelae. Diagnosis of CNS infections and identification of the causative agents requires a complex combination of laboratory tests. In 2010, a hospital-based surveillance study was initiated in Tbilisi, Georgia to determine the incidence of infectious etiologies of acute meningitis and encephalitis, and to enhance laboratory capacity for the diagnosis of CNS infections. Cerebral spinal fluid (CSF) and acute and convalescent sera were collected for bacterial culture and real-time polymerase chain reaction (RT-PCR) testing for herpes simplex virus (HSV) types 1 and 2, mumps virus, enteroviruses, varicella zoster virus (VZV), *Streptococcus pneumoniae*, Haemophilus influenzae type B (Hib), and *Neisseria meningitidis.* Testing for West Nile virus (WNV), tick-borne encephalitis virus, and Eppstein-Barr virus (EBV) was conducted via ELISA. As of April 2012, 144 patients were enrolled. Of these, 44% were adults and 56% were children < 18 years of age. Female to male ratio was 1:1.14. The majority of the patients (75%) were from urban Tbilisi. In 89.7% of enrolled patients, the discharge diagnosis was meningitis and in 8.8% it was encephalitis. Of the meningitis cases, bacterial meningitis was the discharge diagnosis slightly more frequently than viral meningitis (52.8% and 43.4%, accordingly). *S. pneumonia* was cultured from CSF in five patients. One of the secondary study objectives was to measure the occurrence of HiB *following* the initiation of a nationwide vaccination campaign that began in January 2010, shortly before the initiation of this study. None of the patients were positive for HiB. In 140 CSF samples tested by PCR, enterovirus was the most frequently detected etiology (26%). There were three cases of VZV, one case of HSV-1, and two cases of EBV. Data from this ongoing hospital surveillance study provides valuable etiologic and epidemiologic information regarding viral and bacterial acute meningitis and encephalitis in Georgia.

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THIRD CASE OF FATAL YELLOW FEVER VACCINE-ASSOCIATED VISCEROTROPIC DISEASE IN A YOUNG PERUVIAN WOMAN

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Until 2001, yellow fever vaccine was considered to be the world's safest live vaccine. Since then 65 cases of Yellow Fever Vaccine-Associated Viscerotropic Disease (YEL-AVD) have been reported. YEL-AVD resembles yellow fever itself and is frequently fatal. The incidence based on a passive reporting system is 0.3 to 0.4/100,000. However, following an immunization campaign in Peru an incidence of 7.91/100,000 was observed. Known risk factors include age ≥60 and thymectomy as treatment for thymoma. More recently, women of childbearing age have been described as being at increased risk. Nine cases, all fatal, of YEL-AVD in young women have been reported including two from the above Peruvian campaign. Here we describe a third fatal case in a 24 year old Peruvian woman who was vaccinated in preparation for a trip to Australia. The incubation period, day of death following vaccination, and clinical course with multi-organ failure are similar to the other nine cases. RT-PCR demonstrated virus with 100% homology to vaccine virus in serum obtained on the 10th day post-vaccination. The viral load was 45 000 PFU/mL. Of interest is the evidence for concomitant leptospiral infection, lymphopenia, and family history suggesting that the death of a 2 year old male sibling was from meningo-encephalitis. The reasons for the apparent concentration of cases in young women in Peru are unclear. There was no apparent common ethnicity in the three young women. A genetic defect affecting immunity is a plausible explanation and should be investigated with additional research.

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UNDERSTANDING TRICHIASIS FROM THE PERSPECTIVE OF THE PATIENT: AN ASSESSMENT OF PREVIOUSLY OPERATED AND NEVER OPERATED PATIENTS TO IMPROVE QUALITY AND EFFICIENCY OF SURGICAL SERVICES IN ETHIOPIA, NIGER AND MALI

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Ethiopia, Niger, and Mali comprise 26% of the trichiasis burden in the developing world and reported 65% of the global trichiasis surgical output in 2009. In 2011, a study was conducted in each country to understand the trichiasis experience from the patient's perspective with the aim of improving the quality and efficiency of the surgical delivery system. In each country, districts were ranked according to surgery output over the past two years and villages selected at random from the top quartile. Within selected villages all operated and un-operated patients were interviewed to a maximum of 25 and additional villages visited until 192 operated patients had been interviewed. A pre-tested standardized questionnaire asked about demographics, knowledge of trichiasis treatments, health seeking behavior, and perception of surgery; a clinical eye exam was conducted by a trichiasis surgeon. A total of 683 operated and 227 never operated cases were interviewed: Ethiopia 296 and 120; Niger 193 and 35; and Mali 194 and 72, respectively. Among those previously operated, the most common reasons for having surgery were pain, fear of vision loss, and inability to work. Post-operative trichiasis was found in 28.7% of patients in Ethiopia, 33.7% in Niger, and 26.4% in Mali; most had minor trichiasis (<5 lashes). Most patients reported satisfaction with the surgery: Ethiopia, 86%; Niger, 93%; Mali, 92%; most had recommended the surgery to others, reported improvement in vision, and no longer felt pain in the operated eye. Of those never operated, knowledge of the opportunity to receive surgery ranged from 25.0% to 73.5%; however, over 80% reported they had never presented for surgery. Major trichiasis (>5 lashes) was common: Ethiopia, 34.5%; Niger 47.1%; Mali 36.1% . The majority had lived with trichiasis for three or more years and reported pain. Study results will be used to increase community mobilization and awareness about trichiasis surgical services, boost surgical access, improve the quality of surgery, and enhance the overall efficiency of the surgical delivery system.

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PATTERN OF ACUTE POISONING AND PREDICTION OF MORTALITY: A HOSPITAL BASED SURVEY IN DHAKA, BANGLADESH

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Poisoning is a major cause of morbidity and mortality worldwide. Different substances have variable degrees of toxicity from harmless to fatal. Treating physicians often do not know which poison has been taken. It is important to identify life threatening cases to priorities care appropriately. Few systematic investigations of poisoning have been done in the tropics. A large number of poisoning cases remains unreported due to lack of information and awareness at community level. This study collected baseline information and outcome of poisoning in Dhaka, Bangladesh. All cases of poisoning from 1/4/08-30/3/09 admitted to Dhaka Medical College Hospital (DMCH) were recruited for detailed observation. Details of clinical presentation, social background and outcome were recorded. In total, 5932 cases of poisoning in DMCH were enrolled of which 2108/5929 (35%) were female. Median age was 25 years (IQR 19-35 years). Major substances were Benzodiazepines (12%), including deliberate/"induced", organophosphates/carbamates (OPC) (12%), snake/fish/insect and medications. 36% took unknown substances. Suicidal attempt due to family disharmony was the commonest motivating cause (38%). Overall mortality was 151/5932 (2.6%) with 105/151 (70%) of deaths due to OPC (mortality 16%). Other causes of fatal poisoning included benzodiazepines, rat killer, animal/insect bites and stings, methanol, ethanol, herbal medicine and copper sulfate. Risk factors for mortality by univariate analysis were rural abode, hindu religion, illiterate, farmer, suicide attempt, accidental poisoning, GCS<9, BP<90/60mmHg, HR>100 or <60 bpm and abnormal pupils. Multivariate analysis found GCS<11 to be the best predictor of death with the addition of constricted pupils and non-muslim religion in the OPC group and systolic BP<80mmHg, economic loss/failure to pass an exam in the non-OPC group. A simple scoring system was derived using GCS and BP to predict mortality due to causes other than OPC. Poisoning is a common cause of medical admission in Bangladesh. A wide variety of substances are used. OPC poisoning is common and causes two thirds of the deaths. Those at high risk of fatal poisoning can be predicted from history and examination findings.

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RANDOMIZED, DOUBLE-BLINDED, PHASE 2 TRIAL OF WR 279,396 (PAROMOMYCIN AND GENTAMICIN) FOR THE TREATMENT OF CUTANEOUS LEISHMANIASIS CAUSED BY LEISHMANIA PERUVIANA

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In this randomized, double-blind, parallel-group trial, 30 Peruvian patients with parasitologically confirmed cutaneous leishmaniasis (CL) lesions received either WR 279,396 (15% paromomycin + 0.5% gentamicin; n=14) or Paromomycin Alone (15% paromomycin; n=16) topical cream applied once daily for 20 days. Patients were followed for pharmacokinetics (PK), safety, and efficacy for six months. Blood for paromomycin and gentamicin PK parameters was collected from adult subjects after the first days' and last days' drug application. The primary efficacy endpoint was cure of a parasitologically confirmed index lesion, defined as at least 50% reepithelialization of the lesion by Day 63 and 100% reepithelialization by Day 100 with no relapse. At 6 months, final clinical cure of an index lesion occurred in 9/14 (64.3%) subjects in the WR 279,306 group and 11/16 (68.8%) in the Paromomycin Alone group. In pediatric subjects, 9/10 (90%) cured with WR 279,396 and 9/11 (81.8%) with Paromomycin Alone. WR 279,396 appeared to have some benefit over Paromomycin alone as patient's lesions cured at a faster rate when treated with WR 279,396. At 6-months, efficacy of both WR 279,396 and Paromomycin Alone were comparable to historical data for first-line pentavalent antimonial treatments; WR 279,397 cured 5/5(100%) of subjects who had failed prior antimonial therapy. WR 279,396 and Paromomycin Alone creams produced only non-severe application site irritation, without systemic toxicity. PK data showed that there is limited paromomycin and gentamicin systemic absorption thus avoiding drug accumulation and toxicity. Either WR 279,396, or Paromomycin Alone may offer an advantage over first-line antimonial therapies for Peruvian CL, especially in children.

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TRICHIASIS SURGEON PRODUCTIVITY IN ETHIOPIA, MALI AND NIGER: WHAT IS NEEDED TO REACH ELIMINATION BY 2020?

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WHO, donors and NGOs are committed to supporting National Trachoma Programs reach their elimination goals by 2020. Trachoma programs train mid-level eye care personnel and general healthcare workers to perform trichiasis surgery during routine services and outreach campaigns. Although much effort has been put into training surgeons, few studies have examined their productivity. In 2011, a standardized questionnaire was administered by phone to currently active surgeons in Ethiopia, Mali and Niger. The questionnaire asked about demographics, work facilities, supervision, training, and surgeries performed the previous year. In addition, an external ophthalmologist collected qualitative data on selection of trainees, training and supervision. A total of 445 surgeons were interviewed: 191 in Ethiopia, 60 in Mali and 194 in Niger; the majority were male (71%). Most surgeons in Ethiopia were trained <5 years ago, while in Mali and Niger, the majority were trained ≥5 years ago. While nearly 75% of Ethiopian surgeons had been retrained, 1% in Mali and 9% in Niger had been. The mean number of surgeries performed per surgeon in 2010 was: Ethiopia, 76.4; Mali, 156.4; and Niger, 55.9. Most surgery was conducted in outreach: Ethiopia, 93%, Mali, 91%; and Niger 67%. Factors significantly associated ($p < 0.05$) with high productivity in Ethiopia included a higher proportion of time dedicated to eye care, no supervisory visit in the previous 6 months, and increasing years since training. In Mali, the only significant predictor was an increasing number of years since training, whilst in Niger predictors included percentage of time dedicated to eye care, being hospital-based, increasing years since training, and higher number of surgeries performed during training. Study results support that training dedicated eye care workers, improving the frequency and quality of supervision, providing refresher training, and

providing more outreach opportunities will support high productivity among trichiasis surgeons to enable national programs to meet their trachoma elimination targets.

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CUTANEOUS LEISHMANIASIS AND THE EFFICACY OF AZOLES, A SYSTEMATIC REVIEW

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Cutaneous Leishmaniasis (CL) is endemic to 70 countries with 1.5 - 2 million new cases occurring annually. The majority of cases occur in the Middle East (Old World) although the disease is endemic to parts of South America (New World) as well. Travelers and military personnel are at significant risk of acquiring the disease. Its disfiguring and ulcerative lesions result in a high degree of morbidity for those infected. Despite the wide spread distribution and number of cases, debate persists with regard to the most effective treatment for CL. Antimonials have been the mainstay of therapy, but toxicity and constraints with administering the drug make it difficult to use. In recent years, evidence has been building regarding the efficacy of azole antifungals. In order to better characterize the effectiveness of azole antifungals we conducted a systematic review for azole treatment of CL. The databases used were MEDLINE, EMBASE, and Cochrane Database of Systematic Reviews. Search terms included "cutaneous leishmaniasis," "skin leishmaniasis," "therapy," "treatment," "fluconazole," "posaconazole," "ketoconazole," "itraconazole," and "voriconazole." The references from primary studies, narrative reviews and systemic reviews were reviewed to search for additional primary studies that could have been missed by the electronic search. Two investigators independently screened all citations by title and abstract and made a decision on acceptance. Disagreements were resolved by a third author. Inclusion criteria included a confirmed diagnosis of CL, monotherapy with an azole, availability of azole dosage and duration, at least 2 months of follow-up, and at least 4 patients per study. The results of the systematic review will be presented including a breakdown of effectiveness and side-effects of each azole. The aggregated data supports certain azoles as a choice for treating CL. An alogorithmic approach to the treatment of CL is provided.

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BURDEN OF SEVERE DISEASES IN THE FIRST THREE YEARS OF LIFE AMONG A BIRTH COHORT OF 1198 CHILDREN BETWEEN AGES 14 WEEKS AND THREE YEARS IN NORTHERN GHANA

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Reducing under-five mortality by two thirds between 1990 and 2015 according to the Mellenium Development Goals is still quite elusive and efforts to control the cause of the deaths, namely, pneumonia, diarrhea, malaria and malnutrition are being revitalized. Most of these deaths occur in areas with difficulty in gathering accurate data and estimates are the only available means of assessing progress. This study assesses the incidence of serious illness episodes among children aged 14 weeks to 3 years in a cohort of 1198 children recruited into a meningitis vaccine trial in the Kassena Nankana districts in northern Ghana from November 2008 to March 2011. Surveillance teams were set up in all the communities from where children were recruited as well as the health facilities. Serious illness was assessed according to good clinical practices and according to standard clinical criteria. A substantial number had more than one episode of illness during the period under study. Serious illness events were seasonal with over 95% due to infectious diseases. For incidence of severe illness between 14 weeks of age and 3 years, 38.3% of participants experienced at least one episode and the major causes were malaria, acute gastroenteritis and pneumonia which were 20.2%, 7.4% and 2.1% respectively. Proportion of participants who experienced at least one episode of severe illness in the first and second years of life was respectively 130(10.9%) and 175 (16.2%). In the third year of life, 103(9.7%) of participants recorded severe illness and main cause was only malaria with the others being minimal. In all 21(1.8%) of participants died over the 3 years and the main causes of death were respiratory tract infections, malaria and acute gastroenteritis This study confirms a huge burden of preventable infectious diseases among this young age group, where more has to be done in terms of prevention. The relatively low resultant mortality presents a ray of hope that while putting in measures to prevent illnesses, affordable and effective services could be provided to control mortality in children in these age groups in areas of low illiteracy and resource constraint.

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MULTILEVEL ANALYSIS OF TRICHIASIS AND CORNEAL OPACITY IN NIGERIA: THE ROLE OF ENVIRONMENTAL RISK FACTORS ON THE DISTRIBUTION OF DISEASE

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The distribution of trachoma in Nigeria is spatially heterogeneous, with the prevalence generally decreasing in a North-South gradient across the country at a larger scale and more local variation observed within these areas. Relative contributions of individual and environmental risk factors to the geographic distribution of disease remain largely unknown. The primary aim of this analysis is to assess the relationship between climatic factors and trachomatous trichiasis (TT) and/or corneal opacity (CO) due to trachoma in Nigeria, while accounting for the effects of clustering and risk factors at other levels. In addition, we explore the relative importance of clustering at different levels and the respective role of individual and environmental factors on these outcomes. Data from the 2007 National Blindness and Visual Impairment Survey were used for this analysis, which included a nationally representative sample of adults aged 40 and above. Data were available from 305 clusters selected using a multistage stratified cluster random sampling strategy. A basic eye examination was given to all participants and the presence or absence of TT and CO recorded. In addition to field-collected data on individual-level variables, remotely sensed climatic data were extracted for each cluster and used to fit Bayesian hierarchical logistic models to disease outcome. As expected, clustering was apparent at both levels in the model and there was evidence that climatic factors independently contribute to increased risk of TT/ CO after accounting for available individual level risk factors. Beyond some well established individual risk factors (age, gender and occupation), there was strong evidence that environmental factors at the cluster-level (aridity, precipitation and global land cover) were also associated with the prevalence of TT/ CO. This study establishes the importance of large scale geographical risk factors for later stages of trachoma, which confirms anecdotal evidence that environmental conditions are associated with increased risk of these outcomes, and highlights potential uses of risk mapping to better estimate their burden.

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DEMOGRAPHIC AND CLINICAL RISK FACTORS FOR LASSA FEVER IN SIERRA LEONE

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Lassa Virus (LASV) is the etiologic agent of Lassa fever (LF), an acute and frequently fatal illness endemic to West Africa. Multimammate rats (*Mastomys natalensis*) are the reservoir and are found in abundance throughout sub-Saharan Africa. LF is hyperendemic in the Eastern Province of Sierra Leone where humans are thought to acquire infection via exposure to rodent excreta. The National Lassa Fever Surveillance Program of Sierra Leone is headquartered at the Kenema Government Hospital in Kenema, Sierra Leone. All patients who present to the Lassa Fever Ward and meet criteria for suspected LF cases are evaluated using standard forms. Detailed records are kept of presenting signs and symptoms, diagnostic test results for LF, hospital course and outcome. Blood samples from all patients are tested for the presence of LASV NP antigen at the time of presentation by a recombinant-based antigen capture ELISA. Over a 25 month period (January 2010 through January 2012), 1,158 patients presenting to the Lassa Fever Ward met criteria for suspected LF. Ninety-nine (8.5%) patients tested positive for LASV antigen. Patients with an antigen positive ELISA were more likely to present with bleeding, conjunctival injection, facial or neck edema, sore throat, cough, and confusion relative to those with other diseases. In addition, patients presenting greater than 7 days after onset of illness were more likely to have LF. Outcome was known for 96 patients with LF and 230 patients with other diseases. Having LF was strongly associated with a fatal outcome (OR 4.1 CI 2.4-7.0) with a fatality rate of 64%. A fatal outcome was associated with bleeding and presenting seven or more days after disease onset.

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CLINICAL PREDICTORS OF HOSPITAL READMISSION IN UGANDAN CHILDREN WITH CEREBRAL MALARIA

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Cerebral malaria (CM) affects more than 800,000 children each year in sub-Saharan Africa. Determination of clinical symptoms associated with hospital re-admission for children treated for cerebral malaria could help identify those CM children at greatest risk for severe morbidity and mortality. We conducted a study on the pathogenesis of cerebral malaria, and in this substudy, aimed to determine the clinical symptoms associated with greater risk of admission to the hospital in the first 6 months post-discharge in a cohort of Ugandan children aged 18 mo - 12 years. Clinical risk factors were assessed in 165 Ugandan children presenting to the Pediatric Acute Care Unit at Mulago Hospital in Kampala, Uganda, with cerebral malaria who completed 6 months of clinical follow-up. Twenty children (12.1%) were readmitted to the hospital for malaria during 6-month follow-up. Compared to children with CM who were not readmitted, CM children who were readmitted had a higher frequency of measured fever (T≥ 37.5, 85.0% vs. 57.9%; P =0.01) and lactic acidosis (blood lactate > 5.5 mmol/L, 60% vs. 27%, P=0.008) on admission, and were less likely to have received antibiotics during their initial stay at the hospital (55% vs. 80%, P=0.02). Measured fever and lactic acidosis on admission and lack of antibiotics during hospital stay predict risk of readmission in children with CM.

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HEALTH SYSTEM STRENGTHENING THROUGH COMMUNITY REFERRAL IN THE MANAGEMENT OF FEBRILE ILLNESS IN NIGERIA

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Use of Community Health Workers (CHWs) in community case management of febrile illness can improve community-clinic continuum of care, health outcomes and referral system. The main objective of this study is to ascertain the level of home visitation carried out by the CHWs, compliance rate for referrals and treatment response. The authors carried out a record review of 12 months of community registers to ascertain the level of home visitation. To determine compliance to referral, all referral slips and clients' cards at the six primary health care centers participating in the on-going Integrated Community case Management of Malaria were assessed. The CHWs made a cumulative overall home visits of 7,282 to pregnant women 4460 (61.2%) and children under-five years of age 2822 (38.8%). The median visitation for pregnant women was 406 compared to children under-five years of age 257. Overall referral was 578; pregnant women 332(57.4%) while children under-five years of age 246(42.6%). The overall median referral was 28; pregnant women (19) compared to children under-five years of age (9). Overall referral compliance rate was 79.1% (457/578) with pregnant women 73.2% (245/332) compared to children under-five years 86.2% (212/246). Median number of days for pregnant women to comply with referral was 4 compared to children under-five years of age 1.5 days. Reasons for referral for pregnant women, ANC attendance topped the list 78.4 %(192/245); malaria treatment 30.6% (75/245) and reactions to medicines Sulfadoxine-pyrimethamine 2.8% (7/245) and Artemisinin Combination Therapy 3(1.2%) while Children under-five years of age malaria treatment topped the list 60.8% (129/212); diarrhea treatment 23.6% (50/212); pneumonia treatment 14.6% (31/212) and reactions to ACT 0.94% (2/212). All cases were treated same day at the health facility. In conclusion we found relatively high compliance in community referral, and care-givers of children under-five years of age are more likely to comply with referral and very early too than pregnant women. Community health education on referral during pregnancy as a component of case management of febrile illness is recommended for program managers and implementers.

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SIGNS AND SYMPTOMS AS INDICATORS OF FEMALE GENITAL SCHISTOSOMIASIS

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Female Genital Schistosomiasis (FGS) is a neglected, poverty-related disease. Several studies also indicate higher prevalence of HIV in women with FGS. The co-existence of FGS and sexually transmitted infections (STIs) pose a diagnostic challenge for health care providers. The syndromatic management of STIs are strategies in disease prevention in developing countries. In spite of its public health implications, FGS has never been included in any of these protocols. It is therefore important to explore how self-reported symptoms, signs and behavior can be used as indicators of FGS. A school based, cluster randomized, cross-sectional study was conducted in a *Schistosoma haematobium* endemic area in rural South Africa. A total of 921 young women aged 16-22

were included. They were interviewed and asked about symptoms and behavior and gynecological examination with colposcopy was done. Samples (urine, blood and vaginal lavage) for laboratory analyses for STIs and *S. haematobium* were collected. Girls infected with schistosomiasis (cases) were compared with girls without schistosomiasis (controls). Multivariate regression was used for the statistical analyses. Female genital schistosomiasis may be a differential diagnosis to STIs in schistosomiasis endemic areas. It is of importance that health care workers consider this when adequate laboratory facilities are lacking. Symptoms could be added into an algorithm for a syndromatic approach to diagnosis, the meager effect of treatment and reinfection in this age group will be discussed.

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HIV INCIDENCE IN TEENAGE YOUNG WOMEN IN A SCHISTOSOMIASIS ENDEMIC AREA

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Sub-Saharan Africa is severely affected by the HIV (Human Immunodeficiency Virus) epidemic with prevalence higher in women than in men. Young women may be especially prone to HIV infection, but there is still limited knowledge about the reasons for the high prevalence in females. Studies indicate that Female Genital Schistosomiasis (FGS) could be a risk factor. In rural KwaZulu-Natal, South Africa, an area endemic of both *Schistosoma haematobium* and HIV, school-going, sexually active young women were examined twice with an interval of approximately one year. Mean age at first visit was 18.7 years. On both visits the participants went through a detailed interview, including questions regarding water contact, age at sexual debut, alcohol consumption and number of partners. Blood and urine samples were collected, and they were offered a gynecological examination including photocolposcopy. HIV positive and seroconverting young women were compared to the HIV negative individuals. Twenty five high schools of differing schistosomiasis prevalence were included, 921 young women were investigated. Mean age at sexual debut was 16.4 years and 95% reported to have a steady partner when interviewed at first visit. Mean number of lifetime sexual partners was 2 in both groups. The overall HIV incidence was 11.7% and 25% had a genital sandy patches indicating FGS on gynecological examination. The HIV incidence in this schistosomiasis endemic area was unusually high for this age group, however one common risk factor could not be identified. Further analyses for confounders are required. These findings may have implications for the understanding of FGS' role in the different phases of the HIV epidemic.

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COMPUTERIZED IMAGE ANALYSIS AS A TOOL FOR IDENTIFICATION OF CLINICAL MANIFESTATIONS IN FEMALE GENITAL SCHISTOSOMIASIS

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The lesions associated with Female genital schistosomiasis (FGS) consist of changes in the genital mucosa that may be described as sandy patches, characterised by their yellow colour. The gold standard for diagnosing FGS is a biopsy from cervical lesions with direct microscopic inspection for ova. However, this is an inappropriate approach in HIV endemic areas. It is therefore necessary to develop alternative methods for non-invasive, objective diagnosis of FGS that can be performed at the point of care without requiring advanced laboratory equipment or training. The image material was acquired in a study on female genital schistosomiasis in KwaZulu-Natal, South Africa. Healthy individuals were used as negative controls. The mean colour of sandy patches was measured in a subsample of colposcopic images. The colour was represented in a range of colour spaces and compared to the values of the surrounding mucosa using the Wilcoxon signed rank test for paired samples. 7 colour channels were chosen based on the significance level in the Wilcoxon test. The mean differences were used to calculate the most appropriate threshold window in each channel. An algorithm was created in which an image is scored based on presence of pixels present in the intersection of the 7 threshold windows. The validity of the algorithm was tested by running it on a random selection of images in which 3 clinicians had agreed on the diagnosis. It was calculated that 69 pathologic images and as many controls would provide statistical significance when assuming sensitivity of 80% and specificity of 65%. This is a novel method in which computerized image analysis can be used to identify genital schistosomiasis based on the lesions' distinct colour. Further analyses should be done exploring other visual aspects of the lesions, such as morphologic features. It is also necessary to control for confounding factors such as STIs and development is required to adapt this method to socially acceptable clinical practice in a third world setting.

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SUCCESSES AND SHORTCOMINGS OF POLIO ERADICATION: A TRANSMISSION MODELING ANALYSIS

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Polio eradication is on the cusp of success with only a few regions still maintaining transmission. Improving our understanding of why some regions have been successful and others have not will help both with global eradication of polio and with development of more effective vaccination strategies for other pathogens. To examine past eradication efforts we constructed a transmission model for wild poliovirus incorporating waning immunity, age-mediated vaccination rates, and transmission of oral polio vaccine (OPV). The model produces results consistent with the four country categories defined by the Global Polio Eradication Program: elimination with no subsequent outbreaks; elimination with subsequent transient outbreaks; elimination with detected transmission for more than 12 months; and endemic polio transmission. An analysis of waning immunity rates and OPV transmissibility suggest contrasting effects on transmission. Higher waning immunity rates make eradication harder due to increasing numbers of infectious adults. Higher OPV transmission rates make eradication easier as adults become re-immunized. Given these dynamic properties, attention should be given to intervention strategies that complement childhood vaccination. For example, improvement in sanitation can reduce the reproduction number in problematic regions, while adult vaccination can lower adult transmission.

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SUPPORTING THE DEVELOPMENT OF RESEARCHERS IN LOW AND MIDDLE INCOME COUNTRIES IN AFRICA THROUGH PERSONAL DEVELOPMENT PLANNING AND FORMAL MENTORING

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Building a critical mass of researchers in low and middle income countries, who are able to conduct and disseminate high quality research efficiently and effectively, and be able to use results to inform policy and practice in global health is an expectation of funders and institutions. Whilst attention is given to investing in developing the research skills of individuals through fellowship programmes, strengthening systems, infrastructure and institutions, less attention is given to the career planning and

development that is needed to help early-career researchers in these settings become sufficiently established, in order to meet this expectation. Personal Development Planning (PDP) and formal mentoring have been used to support a group of returning African researchers with their career development. The structured and supported process of PDP, assisting in empowering individuals to take ownership of their careers and develop the higher level critical thinking and reflective skills crucial to effective learning, is complemented by formal mentoring where mentors help mentees build confidence towards independence. A participatory action research approach was used to trial and explore how PDP (not used before in this context in sub-Saharan Africa) might help this group of researchers with their career development; in addition to developing an evidence-led PDP model and tools that would work for researchers in Africa. Results showed skills and knowledge gains in research methodology, techniques, communication, networking, updating clinical skills, and developing academic management skills; as well as how these gains were applied effectively in practice. With this same group of researchers, and through a self-selection process of mentees choosing their mentors, a formal mentoring programme was implemented. Whilst mentoring is a long-term process where results and benefits are not always seen immediately, initial results showed that 98% of mentees felt that their mentoring relationship was helping them to progress in their careers, and 85% of mentors were happy with their mentee's progress over the first year. Efforts to make these activities sustainable focus on working with institutions to mentor support groups in PDP and mentoring, and with the aim of embedding these strategies within institutions and programmes. This has the added value of building a network of PDP champions and mentors in Africa.

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GETTING HEALTH CARE DELIVERY RIGHT: LEARNING FROM CASE STUDIES

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The future of global health lies in getting delivery right. Medical schools must prepare the next generation of leaders to confront the management challenges of disease and health programs facing an implementation bottleneck. To aid in this endeavor, we created over 25 teaching cases with accompanying teaching notes offering unique lessons and insights to the principles of global health strategy. From this new body of delivery experience, several themes have emerged: 1) context matters_ programs must account for local factors that influence both the health of the populations and the delivery of health care in their design, implementation, and operations; 2) value_the outcomes achieved divided by the resources invested_is the best measure of program performance, and analysis of value using the care delivery value chain can help program managers determine how best to allocate resources and configure program activities; 3) high-value programs address the social, economic, and geographic barriers to health care delivery; they do not see their objective as "offering" services or technology but as ensuring that the population can realize the full value of the services or technology they are providing; 4) measurement should lead to meaningful learning and program improvement; 5) strategy and leadership are essential as managers face shifts in the landscape and increasing burden of disease. The global health delivery case studies are available free of charge via www.ghdonline.org/cases and provide a tool for educators to build global health delivery competency for the next generation of leaders.

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COMMUNITY HEALTH WORKERS FOR HOME-BASED COUNSELLING AT HOME TO IMPROVE NEONATAL SURVIVAL

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Access to maternal and newborn health services in developing countries is impeded by shortages in human resources for health. We set out to study whether home-based counselling by community volunteers could change home behaviours critical to newborn survival. The objective of the study was to develop and evaluate a community intervention using village-based volunteers to improve newborn care at home. The method included a formative research involving a review of behaviours that impact on neonatal survival, a baseline survey to assess their prevalence, and qualitative work to assess barriers and facilitators of behaviour change. Key messages for home visits in pregnancy and the early newborn period were agreed with stakeholders based on the findings of the formative research, and focus on early and exclusive breastfeeding, clean delivery, and extra care for low birth-weight babies born at home. Newborn foot size was used as a proxy for birth weight to identify low birth-weight babies born at home. In 2010, over 800 volunteers were trained by district health teams, in a randomly chosen 61 of the 131 wards in the 6-district study area. Supervision was improved to involve community leader and nearby health facility staffs. In 2011, a 5,000-household survey interviewed women aged 13-49 about behaviours critical to newborn survival in control and intervention areas. The results showed that over 75% of women in intervention areas were visited by a volunteer at least once in pregnancy, and almost half received a post-natal visit at home. Key behaviours improved as a result of the intervention: tying the cord with clean thread (70% vs 39%, P=0.002), delayed bathing until 6h or more after birth (81% vs 68%, P=0.005), feeding only breast milk for the first 3 days (83% vs 71%, P=0.001), and putting nothing on the cord (87% vs 71%, P<0.001). In conclusion, home-based counselling has improved behaviours critical to newborn survival.

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CLINICAL TRIALS OF THE MEN A CONJUGATE VACCINE CONDUCTED IN WEST AFRICA AND INDIA AMONG INFANTS, CHILDREN AND ADULTS: SHARING ETHICAL CHALLENGES AND LESSONS FOR THE FUTURE

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Application of international ethical guidelines in order to obtain approvals when conducting vaccine trials in diverse local settings can be quite challenging. Since 2005, Meningitis Vaccine Project (a partnership between the World Health Organization and the Program for Appropriate Technologies in Health) in collaboration with the Serum Institute of India has conducted clinical trials on Men A conjugate vaccine across a diverse set of clinical trial sites located in sub-urban and rural communities in India, Mali, The Gambia, Ghana and Senegal. Our collaboration with international, national and local ethics review committees led to the accumulation of huge expereinces on ethical research practices covering aspects of protocol approvals, language and communication in informed

consent, establishing processes for pregnancy testing, supporting health care, obtaining permission and providing feedback to participating communities.

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ENHANCING ACCESS TO MEDICINES THROUGH INNOVATIONS IN WORKING CAPITAL FINANCING FOR DRUG SHOPS

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Access to working capital within the different layers of the healthcare distribution network is one of the factors that limit widespread availability of key medicines and also hampers the sustainability of accredited medicine retailers in sub Saharan Africa. In OECD countries a well functioning credit provisioning system exists across the multiple entities involved in pharmaceutical distribution. Such a credit provisioning system is currently lacking in the private sector pharmaceutical distribution networks in low-income countries. The Accredited Drug Dispensing Outlets (ADDOs) in Tanzania is a very successful model for improving access to high quality medicines and similar models are now being planned in Uganda, Zambia and other countries. To ensure greater sustainability of the accredited drug shop business model and to further enhance its ability to increase access to medicines, a cross-sectional study design was employed using a comprehensive survey instrument developed for the study population of ADDO and ADS owners. The study assessed the cash-to-cash cycle, stocking practices and role of working capital credit within the accredited drug shop network. Findings revealed that accredited drug shops struggle to offer the most appropriate stock assortment at optimal levels. Analysis suggests that both drug shop owners and public health in the community would benefit from better training of shop owners on inventory and cash management and from the provision of additional working capital to the ADDO owners.

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DENGUE RISK PERCEPTION AND BEHAVIORAL RESPONSES BY LOCAL MEMBERS IN DHAKA, BANGLADESH

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Since 2000, there has been a resurgence in dengue virus in the major cities of Bangladesh. This study aimed to assess the risk perception and mitigation efforts towards dengue by analyzing entomological and socioeconomic risk factors in 12 wards within the City of Dhaka, Bangladesh. Data included in the analysis are: a) two vector surveys [i.e., pupal surveys conducted in 847 households (monsoon season 2011) and 459 households (dry season 2012)]; b) a socio-demographic survey of 300 households; c) 12 focus group discussions (FGDs) and eight key informant interviews (KIIs); and d) constructed Knowledge Models of experts and lay persons. Competent dengue vectors were detected in >40% and 12% of households during the monsoon and dry seasons respectively. The monsoon and dry seasonal pupal index were 0.40 and 0.33 respectively for the selected 12 wards. Vector indices were significantly higher for *Aedes aegypti* in this study compared to others conducted in Dhaka in the past. There are significant variations in dengue risk perception between lower (low and medium) and higher socioeconomic groups (SEG). The low and medium SEGs are concerned more about day-to-day issues than exposure to dengue whereas the higher SEG considered themselves at higher risk of dengue infection. Perceived risk from exposure to dengue virus was lower in female subjects than males. Also, experts ranked dengue risk at a much lower level than lay persons and experts emphasized the need for stronger institutional measures to control dengue outbreaks. These findings in turn signify the link between disease risk perception and preventive responses. In consideration of significant SEG and gender variations, targeted education campaigns, vector control and community mobilization programs should be formulated to mitigate the risk of dengue in Bangladesh.

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INCIDENCE, RISK FACTORS, AND COSTS FOR HOSPITALIZATION OF NEONATES BORN TO MOTHERS IN A MATERNAL IMMUNIZATION TRIAL IN BAMAKO, MALI

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Low birth weight (LBW) and prematurity are major causes of neonatal and infant morbidity and mortality worldwide, threatening the achievement of Millennium Development Goal 4. We present the incidences and hospitalization rates of LBW and prematurity among a cohort of infants born to mothers enrolled in a maternal immunization trial, as well as the first cost-analysis of hospitalizations among LBW and/or premature neonates in Bamako, Mali. Women recruited from antenatal care clinics during the 3rd trimester and enrolled after obtaining informed consent were randomly allocated to receive inactivated influenza vaccine (Vaxigrip™, Sanofi Pasteur) or quadrivalent meningococcal conjugate vaccine (Menactra™, Sanofi Pasteur), and followed thru 6 months postpartum. LBW was defined as <2.5kg measured with an infant scale prior to discharge from the maternity center. Prematurity was defined as gestational age <37 weeks by 1st trimester ultrasound if available or otherwise a Ballard exam in the first 7 days of life. All neonatal hospitalizations were identified by 24-hour surveillance. Direct and indirect costs incurred by any hospitalized neonate meeting definitions for LBW or prematurity were recorded daily. From September 2011 thru March 2012, there were 652 liveborn infants, of whom 3.4% were premature and 10.9% were LBW. The rate of hospitalization or death during the 1st month of life was 4.0% among all infants, compared to 17.7% for newborns <2.5kg (RR: 8.2, 95% CI: 3.9-16.8) and 36.4% for newborns <37 weeks (RR: 13.4, 95% CI: 6.3-26.2). Complete cost data were available for 22 hospitalized infants, of whom 14 were LBW and premature, 7 were LBW only, and 1 was premature only. The median duration of hospitalization was 7 days (IQR: 5-11), with a median cost of 94.44 USD (IQR 56.43-157.25). Direct expenditures accounted for 79% of all costs, with medication purchases responsible for the majority. In addition to mortality, prematurity and LBW cause substantial morbidity and economic hardship for families in Mali. Interventions that can reduce the risk for prematurity or LBW are urgently needed.

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CAN COMMUNITY HEALTH WORKERS PROVIDE QUALITY INTEGRATED COMMUNITY MANAGEMENT OF FEBRILE ILLNESSES: A CASE STUDY OF COMMUNITY HEALTH WORKERS IN TWO SELECTED LOCAL GOVERNMENT AREAS OF AKWA IBOM STATE, NIGERIA

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The World Health Organization has recommended improved quality of care as key elements in strengthening health systems in poor resource countries, Engagement of Community Health Workers (CHWs) can reduce challenges such as weak public sector, human resource constraints, and variable quality of the private sector. Efforts to improve access to quality case management of febrile illness in Nigeria included the engagement of Community Health Workers (CHWs) to use Rapid Diagnostic tests as a component of home management of malaria, dispense ACTs and manage pneumonia and diarrhea. This current effort monitored and measured the performance of CHWs in providing quality management of febrile illnesses in two selected LGAs. The authors trained one hundred and fifty-two CHWs and developed simple quality performance standards (one-page tool) for CHWs providing community services in Akwa Ibom State, Nigeria. All 152 trained CHWs providing malaria, pneumonia and diarrhea case management were monitored and assessed using the standards. The tool has 37 performance criteria (PC) to measure CHW knowledge, skills and competence in 3 sections: History taking and Examination; Conducting RDTs for Malaria; and Illness Management. Trained assessors observed CHWs providing services. Each correctly performed criterion was scored 1 point. Four rounds of assessments were conducted at an interval of two months from June 2011 - March, 2012. During Round 1 CHWs achieved an average of 19 (52.2%) PC. This rose to 25 (67.5%) PC at Round 2; 28 (75. 6%) at Round 3 and 30 (81.1%) and ($p = 0.00$). PC that needed most improvement included reinforcement on checking RDT expiry date, entering results on records, and safe disposing of sharps. CHWs can provide quality case management of febrile illness in the current efforts to reduce annual deaths of people at risk while contributing to the achievement of targets numbers 4, 5 and 6 of the Millennium Development Goals (MDGs). In conclusion CHW supervisors can use this tool to enhance the quality of services provided by the CHWs and improve CHW training.

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REGULATION OF VACCINES TO PROTECT AGAINST GLOBAL INFECTIOUS DISEASES: A ROADMAP TO WORKING WITH THE UNITED STATES FOOD AND DRUG ADMINISTRATION

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Global infectious diseases (GID) such as tuberculosis, malaria, dengue and hookworm affect more than one billion people worldwide. The development of safe and effective vaccines for the prevention of these diseases is of critical importance not only for global humanitarian reasons but also for United States (U.S.) public health. The submission of an investigational new drug application (IND) for a vaccine or biologic to the US FDA can provide sponsors with important scientific and regulatory advice on products that are critical to the advancement of world health. If pursued, U.S. licensure signifies to the global medical and regulatory community that the FDA has made the determination that the vaccine is safe and effective. This finding by the FDA may assist other National Regulatory Authorities in their evaluation of the vaccine. The US FDA recently updated the Guidelines for the Development of Vaccines to Protect Against Global Infectious Diseases. This presentation aims to introduce vaccine developers to these recently updated recommendations and to the regulatory review process at the Division of Vaccines and Related Products Applications (DVRPA) in the Office of Vaccine Research and Review at the Center for Biologics Evaluation and Research (CBER), U.S. FDA. The following issues will be discussed: acceptability and utility of non U.S. studies to support product licensure; the use of clinical bridging studies and how these data may be used to determine interregional acceptance of foreign data; safety monitoring during international vaccine clinical trials. Regulatory issues in the manufacture and pre-clinical testing of new vaccines for global health will be presented. Finally, general principles pertaining to evaluation of vaccine safety and effectiveness, and common concerns related to vaccine manufacturing submissions will be reviewed.

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THE WORLD INTELLECTUAL PROPERTY ORGANIZATION (WIPO RE:SEARCH) PARTNERSHIP HUB: GENERATING NEW COLLABORATION OPPORTUNITIES TO ACCELERATE NEGLECTED TROPICAL DISEASE R&D

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Collaborations are a key mechanism to more effectively and efficiently discover and develop new drugs, vaccines, and diagnostics to help the more than 1 billion people suffering from neglected tropical diseases (NTDs), malaria, and tuberculosis. Recognizing the need for more progress in neglected disease research, the WIPO Re:Search Consortium was formed in October 2011. The World Intellectual Property Organization (WIPO) in partnership with BIO Ventures for Global Health and several of the world's leading pharmaceutical companies, renowned academic and other neglected disease research organizations provide access to intellectual property for pharmaceutical compounds, compound libraries, technologies, and importantly expertise and knowledge to support research and development for NTDs, malaria and tuberculosis. WIPO Re:Search aims to expand the number of drug, vaccine, and diagnostic technology candidates for NTDs by sharing these valuable resources and knowledge to accelerate product development. The WIPO Re:Search Partnership Hub facilitates research collaborations among WIPO Re:Search members by fielding requests for specific targets or compounds of interest, identifying collaboration opportunities among key biopharmaceutical and neglected disease research institutions, and providing scientific expertise to proactively match contributions in WIPO Re:Search with members' research program needs. The Partnership Hub establishes mutual interest in exploring a collaboration opportunity and then connects members so that scientists can discuss their research and collaborate. This presentation will focus on the WIPO Re:Search Partnership Hub as an innovative model in global health. We will explain how the Hub has facilitated successful collaborations, and will highlight the impact that the Partnership Hub model has had in accelerating product development for NTDs, malaria, and tuberculosis.

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THE DRUG DRUG INHIBITION POTENTIAL OF ANTI-MALARIAL AGENTS

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Therapeutic regimes for malaria involve the co-administration of two or more compounds. The patient can also have additional therapy of which HIV therapy is an example. These combinations have the potential to cause drug-drug interactions (DDIs) leading to drug exposures that vary from that intended. This can be critical when determining combination partners and if the drugs in question have narrow therapeutic indices. A weakness of published IC_{50}s is that they are dependent on experimental

conditions which are often not comparable or able to be transformed into Ki values. The inhibition of the following CYP450 isoforms - 1A, 2B6, 2C8, 2C9, 2C19, 2D6, and 3A4 - were investigated using the metabolism of their specific substrates. Mefloquine, piperaquine, pyronaridine, OZ439, naphthoquine, dihydroartemisinin, primaquine, amodiaquine, chloroquine and lumefantrine were the marketed or MMV proprietary drugs nvestigated. They were investigated in concentration response experiments where the test compound (0.1 µM - 25 µM) was incubated with human liver microsomes and NADPH in the presence of a cytochrome P450 isoform-specific probe substrate. Potent inhibition was considered as IC_{50} < 1 µM, moderate inhibition was considered as IC50 between 1 and 10 µM, and no or weak inhibition was considered as IC_{50} > 10 µM. All of the isoforms tested were inhibited by at least one of the test compounds except for 2C9 which was not inhibited. The majority of the inhibition observed was determined to be moderate except for 1A where primaquine caused potent inhibition. The most affected isoform was 2D6 (4/10 compounds inhibited). Piperaquine, OZ439 and lumefantrine did not cause any inhibition of the isoforms tested. This data can be used as a first guide in forming antimalarial combinations and when combining several therapeutic approaches. Further work will be done to augment these results. This will include widening the set of compounds tested, determining the metabolic pathways of the pathways tested and determining Ki values for inhibition where the IC_{50} values warrant it.

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MATHEMATICAL MODELING OF THE EFFECTS OF DRUGS ON MALARIA TRANSMISSION IN LOW TRANSMISSION AREAS

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Malaria eradication is now the ultimate objective of many organizations, including the Roll Back Malaria Partnership, the Bill and Melinda Gates Foundation, and the Global Fund. Achieving this objective will require utilizing the three pillars of malaria control: insecticide-treated bed nets, spraying, and antimalarial drugs. However, the impacts of drugs on malaria transmission are not yet fully understood. We describe the development and application of a new mathematical modeling framework to simulate the effects of dug treatment on transmission in low transmission settings. We find that the addition of gametocytocidal drugs to standard treatment regimens may play an important role in reducing transmission. However, we find that the reductions are not large when only symptomatic individuals are treated. Further, the reductions in transmission from the addition of gametocytocidal drugs can be achieved by other means, such as increasing the fraction of individuals treated and reducing the time to treat. These three methods of reducing transmission can be combined, depending on context of malaria transmission in an area. We also present preliminary results using our modeling framework to predict the effects of artemisinin resistance on treatment outcomes with a variety of artemisinin-based therapies.

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HEMATOLOGIC COMPLICATIONS AFTER INTRAVENOUS ARTESUNATE IN PATIENTS WITH SEVERE MALARIA

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Fast acting anti-malarials are essential to treat severe malaria. Existing evidence shows that intravenous artesunate is significantly more effective in parasite clearance but also with respect to survival compared to quinine. Clinical benefits of artesunate appear to be most prominent in patients with high parasitemia. However, previously unknown complications like delayed hemolysis has been described in an increasing number of patients with imported severe malaria. There appears to be an association of post-treatment hemolysis with high parasite levels. We have seen similar hematologic complications after treating three patients with imported severe malaria during a prospective follow-up in our center in Hamburg, Germany. Post-treatment hemolysis occurred in all patients and reached its peak around 14 days after initiating intravenous artesunate. In addition to signs of hemolysis like a second rise in LDH levels, there was a low reticulocyte production index in all patients indicating prolonged impairment of erythropoiesis. We are currently investigating this relevant complication in children with severe malaria in Africa. In addition, pathophysiologic analyses including murine models are underway. Evidence of post-treatment hemolysis, potenial pathogenesis and clinical relevance both for imported as well as endemic severe malaria are discussed.

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TRENDS IN U.S. MILITARY HEALTH SYSTEM (MHS) MALARIA CHEMOPROPHYLAXIS PRESCRIBING PATTERNS FROM 2007-2011

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No systematic reviews describing malaria chemoprophylaxis prescription trends within U.S. primary care settings have been conducted. The MHS, with its 9.7 million beneficiaries, represents an enormous pool of potential travelers to be considered for malaria prevention measures. Military force health protection policies target active duty forces but may affect all beneficiaries if providers implement policy guidance comprehensively. In 2009, the Department of Defense released malaria prophylaxis policy guidance limiting the use of mefloquine in deployed personnel. A systematic search of the MHS electronic medical record was performed for all prescriptions of atovaquone-proguanil (AP), chloroquine (CQ), doxycycline (DC), and mefloquine (MQ) to adult patients from 2007-2011. For CQ and DC, search parameters were filtered to target malaria chemoprophylaxis. Absolute and proportional prescribing rates for the total, active duty (AD) and dependent/retiree (DR) populations were assessed for changes over time. Trends for prescriptions originating from primary care (PC) clinics versus specialty travel (ST) clinics were also compared. A total of 624,416 prescriptions (AP 7%, CQ 3%, DC 76%, MQ 14%) were identified during the study period, including 156,150 DR patients (25%). Prescription volume rose from 64K (AP 11%, CQ 8%, DC 45%, MQ 36%) in 2007 to 180K (AP 6%, CQ 1%, DC 89%, MQ 4%) in 2011, with DC representing the majority of the increase ($p<0.001$). MQ use diminished in all clinics over time. Whereas ST clinics predominantly and increasingly prescribed AP (58% in 2007 and 71% in 2011), PC clinics predominantly and increasingly prescribed DC (54% in 2007 and 96% in 2011). Trends were similar for AD and DR populations, suggesting that health policies influence prescription practices in both groups. This study is the first longitudinal systematic review of malaria chemoprophylaxis patterns in the U.S. adult population. Health policies and provider specialty influence malaria chemoprophylaxis choices.

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MALARIA KNOWLEDGE AND USE OF MALARIA PREVENTION IN THE UNITED KINGDOM POPULATION AND BY UNITED KINGDOM TRAVELERS TO MALARIA ENDEMIC COUNTRIES

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Few data exist on travellers' knowledge and practices regarding malaria and its prevention, or the numbers of travellers receiving advice or taking chemoprophylaxis when visiting malaria-endemic areas. This study was undertaken to evaluate British adults' knowledge of malaria, and utilisation of anti-malarials through a face to face questionnaires. Two groups were surveyed; a sample of Great Britain's adult population through an IPSOS MORI's Capibus survey of 1,991 adults aged >=15 years old, of whom 548 had previously visited a malaria-endemic country. The 2nd group were 500 passengers in departure areas of Heathrow

Airport departing to a malaria endemic areas, by the airport authority (CAA). All were questioned about their malaria knowledge and used of prophylaxis and other measures. 40% were travelling to W Africa and 38% East and Central Africa. Knowledge and advice scores based on respondents' knowledge of symptoms, seriousness, curability of malaria were calculated. The IPSOS cohort's mean knowledge score was 3.21, versus 2.98 in non- travelled (n=548 & 1443), p<0.001 with a similar score in the CAA travellers of 3.23. The source of advice obtained was categorized scored as a) professional and b) non-professional or no advice. Most had had obtained professional advice - 55% of IPSOS and 61% of CAA travellers - while most travellers not using prophylaxis had not. Prophylaxis use was reported by 77% of Kenyan, 81% of Ghanaian and 49% Nigerian departing passengers. In the CAA travellers, mean knowledge score was similar in those who used prophylaxis or not (3.3 and 3.2 respectively) and the same was true in the IPSOS travellers (mean 3.2 whether used prophylaxis or not). Statistical analysis will be presented of chemoprophylaxis and other factors which may be associated with knowledge of malaria.

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DISCOVERY OF A NOVEL TARGET FOR ANTIMALARIAL THERAPY: CYTOPLASMIC PROLYL TRNA SYNTHETASE IS THE TARGET OF HALOFUGINONE IN *PLASMODIUM FALCIPARUM*

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Many current anti-malarial drugs work within the same biological pathways leading to shared resistance mechanism. We have taken the methodology of chemogenomics to identify potential antimalarials that target novel pathways. Understanding the anti-plasmodial mechanism of halofuginone (HFG), a febrifuginone analogue, informs our understanding of parasite biology and directs the future creation of novel therapies. To interrogate mechanism, we selected parasites that are resistant to halofuginone and then used whole genome sequencing to identify the causative mutation (SNPs) and developed high resolution melting (HRM) genotyping assays to follow up those most promising. We found two nonsynonomous mutations in the active site of the cytoplasmic prolyl-tRNA synthetase (*Pf*cPRS) in independent selections. Using a heterologous *S. cerevisiae* model, we have confirmed the sufficiency of *Pf*cPRS to confer sensitivity to halofuginone. In addition, the two nonsynonous SNPs abrogate sensitivity to halofuginone. Amino acid deprivation of *Plasmodium falciparum* activates the amino acid starvation response (AASR) – a highly conserved stress pathway that inhibits cell-wide translation. To determine the involvement of AASR, we then performed western blot analysis of the phosphorylation of eukaryotic initiation factor 2 α (eIF2α) in the presence or absence of excess proline. We have found that halofuginone and febrifugine block *P. falciparum* proline metabolism. Treatment of parasites with halofuginone and febrifugine also results in increased phosphorylation of a *P. falciparum* eIF2α analogue. Furthermore, proline supplementation in the media decreases sensitivity to halofuginone in a dose-dependent fashion. In a similar dose dependent manner, the phosphorylation of eIF2α is dependent on the level of exogenous proline in the presence of halofuginone. Overall, these results demonstrate halofuginone-induced proline starvation via an interaction with *Pf*cPRS leads to translational inhibition. Thus we posit that the potential of amino acid supply and aminoacyl tRNA synthetases as a new promising and potential target for chemotherapeutic intervention.

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QUANTIFYING THE ANTIMALARIAL MARKET IN AFFORDABLE MEDICINES FACILITY (AMFM) PILOT COUNTRIES THROUGH ANALYSIS OF IMPORT, EXPORT AND LOCAL MANUFACTURING RECORDS

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The Affordable Medicines Facility for malaria (AMFm), an innovative financing mechanism which aims to increase access to artemisinin-based combination therapies (ACTs) on a multi-national scale through an ex-factory subsidy, launched in July 2010. Since the first private sector order was placed in July 2010, over 158 million ACTs have been ordered by private sector buyers across all active AMFm countries. While these figures likely represent a significant increase in ACT volumes procured in the private sector from pre-AMFm years, understanding the market share and implications for national and global drug forecasts requires better understanding of the total antimalarial market size in these countries. In this investigation, a quantification of the antimalarial markets in 7 of the 9 AMFm pilot countries was performed through a top of the supply chain analysis. For each country, antimalarial import, export, and local manufacturing data were gathered and analyzed. The number of treatment doses procured and manufactured over a three year period was combined and exports deducted to estimate the net market size. The results of this analysis describe the antimalarial market, including ACT market share, before and during initial implementation of the AMFm. For example, an initial analysis of imports, exports and local manufacturing records showed that the total market size in Kenya for 2008 was 43.8M: 25.4M in the private sector and 18.4M in the public sector, representing a significant increase from previous antimalarial demand estimates. This analysis provides context for better understanding the impact of the AMFm and offers a baseline for future analysis of antimalarial demand.

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TRACKING MALARIA CASE MANAGEMENT COVERAGE IN THE ERA OF ACT AND RDT SCALE-UP: POSSIBILITIES AND LIMITATIONS WITH USING HOUSEHOLD SURVEYS

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Global malaria control targets focus on coverage of appropriate case management of suspected malaria among children under five. The key indicator for measuring progress towards targets in endemic countries has been presumptive treatment of all fevers among children under five measured using population-based surveys. Access to diagnostic testing and policy change focused on diagnosis before treatment diminish the relevance of the presumptive treatment indicator. This presentation focuses on the value of population-based surveys in the context of scaling up access to treatment and diagnosis. Nationally-representative household surveys focused on treatment-seeking behavior for suspected malaria were conducted in 2012 in Uganda, Madagascar and Nigeria as part of the ACTwatch research program. The timing of these surveys falls at the end of the Affordable Medicines Facility malaria (AMFm) pilot which aimed to increase access to artemisinin-based combination therapy (ACT) in the public and private sectors. Detailed information on treatment-seeking behavior was collected, including where treatment was sought, services and medicines received, and perceived quality of care received at each source. Perceptions regarding dimensions of quality of care were also assessed across local options for fever treatment. Results from questions on type and result of blood testing, and treatment based on test results will be discussed - including issues with respondent recall and respondent awareness of diagnostic test results and treatments received in the context of patient-provider interactions that characterize these settings. While household surveys can provide information on where treatment is sought

and to some extent why, complementary data are necessary to improve case management of suspected malaria and to track progress towards global targets. Methods and measures will be discussed.

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REACH OF THE GREEN LEAF: EXPOSURE, AWARENESS, AND REPORTED USE OF AFFORDABLE MEDICINES FACILITY (AMFM)-BRANDED MEDICINES IN THREE COUNTRIES

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The Affordable Medicines Facility - malaria (AMFm) is a global initiative aiming to expand access to affordable artemisinin combination therapy (ACT). The AMFm seeks to reduce consumer prices through price negotiations and a buyer co-payment for which both public and private first-line buyers at the country level are eligible. Reduced prices are expected to extend down the antimalarial supply chain so that effective medicines are available and affordable for consumers. Changes in household treatment-seeking behavior and improved household fever management are expected as access to effective antimalarials increases. Subsidized quality-assured treatments are marked with a green leaf logo to facilitate product promotion and consumer recognition. The first phase of the AMFm began in 7 sub-Saharan African countries in 2010/11. The ACTwatch research program conducted nationally representative household surveys in 2012 in 3 AMFm countries: Uganda, Nigeria, and Madagascar. The studies investigated treatment-seeking behavior for recent fever in children under five. Questions to assess awareness of the AMFm program and the green leaf logo were administered to caregivers in all households with children under five. In households where children had fever in the past 2 weeks, questions on use of antimalarial medicines included recall of the green leaf logo on drug packaging. Information on type, timing, and source of antimalarial treatments obtained was also collected. Results on the reach AMFm communications and the green leaf logo, and implications for treatment-seeking behavior and treatment outcomes will be discussed.

1301

PRIVATE SECTOR DEMAND AND AVAILABILITY OF ARTEMISININ-BASED COMBINATION THERAPIES UNDER THE AFFORDABLE MEDICINES FACILITY FOR MALARIA

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Phase 1 of the Affordable Medicines Facility for malaria (AMFm), a buyer-subsidy program that aims to increase consumer access to artemisinin-based combination therapies (ACTs), launched across nine countries in 2010 and 2011. This program is likely to have a significant impact on the ability of consumers to purchase previously unaffordable ACTs in the private sector, where many seek treatment for malaria-like illness. As of early April 2012, private sector buyers had procured 158M ACTs through the AMFm mechanism. Despite these substantial ACT volumes, it's unclear how well private sector procurement has met consumer demand. Using a dynamic sub-national market forecasting model based on data collected during routine household surveys along with assumptions drawn from the published literature and ongoing operational research, we forecasted private sector consumer demand for antimalarial medicines, including ACTs, following the launch of AMFm. We validated our model using a 2012 analysis of antimalarial import, export, and local manufacturing records in 7 of the 9 AMFm countries. Finally, we estimated the portion of private sector treatments that are likely used to treat true malaria infections and the average cost per treated malaria episode. Results demonstrate that across AMFm countries in 2012, private sector consumer demand for ACTs is significantly greater (165M) than the projected private sector procurement volumes (83M) through AMFm. The mean cost per ACT-treated malaria episode varied widely across the eight AMFm countries, with highest per-infection ACT costs in regions with low malaria prevalence ($6.37 in Kenya) and the lowest in areas with high prevalence ($2.27 in Nigeria). The substantial demand for ACTs in the private sector in AMFm countries suggests that the ACT subsidy's goal of crowding out inferior antimalarial medicines will be difficult to achieve with current procurement rates. In addition, this study provides an indication of the potential cost savings that could result from implementation of improved diagnostic methods in the private sector.

1302

EFFICACY AND TOLERABILITY OF DIHYDRO-ARTEMISININE-PIPERAQUINE (DUOCOTEXCIN*) VERSUS ARTEMETHER-LUMEFANTRINE (COARTEM*) FOR THE TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN SENEGAL: OPEN RANDOMIZED TRIAL

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Malaria remains a major public health problem in Sub-Saharan Africa. Prompt an effective treatment is essential for malaria control. Malaria treatment requires the use of Artemisinin Combination Therapies (ACT). In Senegal, ACT are widely in health care units. In the context of the scaling up of antimalarial treatment, there is a need to monitor ACT efficacy. The study was carried out from November 2011 to January 2012 in Deggo health center within the district health of Pikine (located at 20 from Dakar the capital city). Study end points included (i) PCR corrected adequate clinical and parasitological response (ACPR) at day 28, (ii) ACPR at days 35 and 42, (iii) parasites and fever clearance time, (iv) incidence of adverse events and patients biological profile at day 7. The WHO 2003 protocol for antimalarial drug efficacy evaluation was used to assess each outcome. Overall, 240 patients were randomized to receive either Dihydro-Artemisinine-Piperaquine (Duocotexcin*) (n=120) or Artemether-lumefantrine (Coartem*) (n=120). PCR corrected ACPR at day 28 was at 93.3% in the Dihydro-Artemisinine-Piperaquine group while that was at 97.5% in the Artemether-Lumefantrine group (p=0.21). Therapeutic efficacy was at 100% in Dihydro-Artemisinine-Piperaquine group verus 99% in Artemether-lumefantrine group at day 35 (p=0.44). At day 42 ACPR at 100% was obtained in the two treatments group. The two treatments were well tolerated with similar clinical and biological profile. Abdominal pain, vomit and dizziness were the most frequent adverse event in two treatment group. No serious adverse event was noted in the two study groups. In conclusion, Dihydro-Artemisinine-Piperaquine (Duocotexcin*) and Artemether-lumefantrine (Coartem*) are still efficace and well tolerated and are suitable for the treatment of uncomplicated *P. falciparum* malaria in Senegal.

1303

EFFECTS OF PLASMA PIPERAQUINE LEVEL ON THE ELECTROCARDIOGRAM IN PATIENTS WITH UNCOMPLICATED MALARIA RECEIVING A TWO- VERSUS THREE-DAY COURSE OF DIHYDROARTEMISININ-PIPERAQUINE IN NORTHERN CAMBODIA

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DHA-piperaquine is currently recommended as a first line treatment for uncomplicated *Plasmodium falciparum* and *P. vivax* in Cambodia, and worldwide (WHO 2010). A post-treatment prophylactic effect of DHA-PIP up to 63 days has been reported, making it potentially valuable in malaria eradication efforts. However, cardiotoxicity is an important potential concern with PIP due to QTc interval prolongation. While a 3-day course is widely recommended, the Cambodian military currently employs a 2-day regimen in order to improve compliance. As part of a clinical trial comparing therapeutic efficacy of 2 versus 3 day dosing regimens of DHA-PIP, cardiac safety was evaluated by comparing plasma PIP levels to EKG results. In an open-label clinical trial, 80 patients developing uncomplicated malaria infections of any species were randomized 1:1 to receive a directly observed cumulative dose of 320mg DHA/2880mg PIP divided into either a 2 or 3 day course as inpatients. Plasma piperaquine levels from all volunteers receiving DHA-PIP were collected at pre-dose, 4, 24, 48, 72 hr, 7, 14, 21, 28, 35 and 42 days after the first dose, and on the day of recurrence. Patients had 12 lead EKGs at screening, pre-dose, daily for 3 days and then weekly for 4 weeks if prolongation was more than 10 ms during dosing. Pharmacokinetic analysis is currently in process. Of 35 out of 80 completed subjects with levels measured by high performance liquid chromatography-mass spectrometry to date, there were 8/23 (34.8%) from the 2-day and 2/12 (16.7%) from the 3-day regimen with a positive correlation between the change in QTcB from baseline and the log of the piperaquine concentration. Final results will be presented to determine if either regimen posed a greater risk for QTc prolongation.

1304

STABILIZING SUPPLY AND AVOIDING NATIONAL LEVEL STOCK OUTS OF ACTS IN AN ERA OF WIDE ACT SCALE-UP

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The global market for ACTs has been fraught with volatile demand due to poor planning and supply chain management, uncertainties in funding, and uncertainties in supply due to the long lead times involved in production and the vagaries of an agricultural starting material. Unless appropriately managed these uncertainties have the potential to disrupt global supply of ACTs and hamper the the scale-up efforts achieved in the last few years. Better matching of demand and supply and building resilience in the ACT supply chain may require new approaches. Policy makers have begun to discuss buffer stocks, volume guarantees and other mechanisms to ensure an uninterrupted supply of ACTs to meet the fluctuating demand. However, to date no detailed and rigorous analysis of these mechanisms has been performed to understand their suitability, benefit and cost effectiveness for the ACT supply chain. This paper attempts to address this issue. Models such as a regional ACT buffer stocks, a buffer capital fund, and minimum volume guarantees to ACT manufacturers are discussed for their effectiveness, efficiency and feasibility for this context.

1305

AN EVALUATION OF TREATMENT RESPONSE TO ARTESUNATE-MEFLOQUINE FIXED-DOSE COMBINATION IN CHILDREN DURING A DEPLOYMENT STUDY IN AMAZON BASIN COMMUNITIES OF BRAZIL

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The World Health Organization currently recommends the use of five Artemisinin Combination Therapies (ACTs) for the treatment of uncomplicated malaria. The Drugs for Neglected Disease *initiative* (DND*i*), together with the Special Program for Research and Training in Tropical Diseases (TDR) and the Drugs Technology Institute of the Oswaldo Cruz Foundation (Farmanguinhos/Fiocruz) developed an artesunate-mefloquine fixed-dose combination (ASMQ FDC). The public health impact of ASMQ FDC was evaluated between 2006-2008 in a total of 23,845 patients in the Amazon basin of Brazil, in collaboration with the Brazilian Ministry of Health and RAVREDA-AMI/PAHO. As a large number of these patients (8880;37,2%) were patients under the age of 14, we decided to conduct a post-hoc assessment of treatment outcomes in this specific patient population in the municipality of Cruzeiro do Sul, in order to gather data on the use of ASMQ FDC in children. Cruzeiro do Sul was selected because it is an urban area in which patient follow-up smears are more readily accessible. A total of 584 patients under the age of 14 presented for a follow-up slide until day 40 - the time defined as the interval for recrudescence by the study protocol. Less than 2% of the originally tested patients (8/584;1,4%) had positive thick smears for the malaria parasite, equally distributed among different age categories. Asexual forms of the parasite were detected in a total of 4 cases (0.68%); among which a case with both asexual and sexual forms. These positive malaria smears could represent either re-infections or recrudescence of the initial infection. Our data represent important additional information on the effectiveness of ASMQ FDC in children, and support its use in this specific population. They are consistent with results of other clinical studies, performed in different epidemiological settings and populations.

1306

CLEARANCE OF *PLASMODIUM FALCIPARUM* AS ASSESSED BY RAPID DIAGNOSTIC TESTS, MICROSCOPY AND PCR FOLLOWING ANTI-MALARIAL TREATMENT IN TANZANIAN CHILDREN

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Rapid Diagnostic Test (RDT) has become an important tool for confirmatory malaria diagnosis. Until recently most tests have been based on detection of Histidine Rich Protein 2 (HRP2), a sensitive and stable marker for *Plasmodium falciparum (Pf)* malaria. However, the usefulness of HRP2 based RDT detection of *Pf* is hampered by persistent antigenemia causing false positivity even after successful treatment. Conversely, *Pf*-specific Lactate Dehydrogenase (LDH) based RDT has been proposed to detect only live parasites. Our aim was to study *Pf* clearance as assessed by these two antigens (HRP2 and LDH) in comparison with microscopy and PCR after artemisinin-based combination therapy in Tanzanian children.

Some 50 children <5 years with uncomplicated *Pf* malaria were enrolled. The children were examined on nine occasions during a 42-day follow-up period. At each visit blood was collected for the two RDTs (ParaHit® and CareStart®), Giemsa and Acridine Orange staining of blood slides for microscopy and filter papers for real time-PCR based detection of parasite DNA. A majority of children cleared their parasitemia ≤3 days as accessed by microscopy and PCR. Median HRP2 and LDH positivity time after treatment initiation was 21(range 3-42) and 3 (range 1-7) days, respectively. Due to the remaining HRP2 positivity, this RDT was unable to identify recurrent malaria infections that occurred during follow-up in 10/50 (20%) of the children, whereas the LDH based RDT identified eight of these recurrent infections. The results suggest LDH based RDTs to be more suitable for *Pf* detection in high endemic areas.

1307

HRP2 AND PLDH RDTS COMPARED WITH MICROSCOPY, PCR AND HISTOLOGY FOR DETECTION OF PLACENTAL MALARIA DURING PREGNANCY AND AT DELIVERY IN AREAS OF VARIED TRANSMISSION

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Malaria prevention measures for pregnant women are critical and available, but the effectiveness of intermittent preventive treatment in pregnancy (IPTp) with sulfadoxine-pyrimethamine (SP) is declining with increasing parasite resistance. Diagnostic testing may allow better targeting of efficacious antimalarial treatment to asymptomatic women with demonstrated malaria infection. Light microscopy of peripheral maternal blood misses a large proportion of cases, and PCR is unavailable in routine care. Early data show that detection of parasite antigen in maternal blood may indicate clinically significant infection and predict pregnancy outcomes. Therefore, screening with RDTs may offer a practical way to identify pregnant women who will benefit from targeted therapy for placental malaria infection. We assessed the detection of asymptomatic malaria infection in pregnancy by highly-characterized RDTs in two African clinical settings (Uganda, hyperendemic, and Burkina Faso, seasonal transmission). We enrolled 995 (345 Uganda, 650 Burkina Faso) HIV-negative women in the second or third trimester of pregnancy and followed them to delivery. On the standard IPTp schedule and at delivery, participants' blood was collected for RDTs detecting histidine-rich protein 2 (HRP2) and plasmodium lactate dehydrogenase (pLDH), malaria microscopy and PCR; placental tissue for histology was obtained at delivery. Participants with negative RDT results received SP; those with a positive RDT received artemether-lumefantrine or quinine, and SP. Preliminary data show that 130 (38%) and 112 (32%) participants were positive by HRP2 and pLDH, respectively, at enrollment in Uganda; 134 (21%) were positive by either RDT at enrollment in Burkina Faso. Quality controlled interpretation of peripheral and placental blood microscopy, PCR and histology samples is on-going. Data will be presented on the accuracy of these diagnostic testing methods for detection of asymptomatic malaria during pregnancy and on the potential utility of RDT screening for management of such infections.

1308

ABSOLUTE QUANTIFICATION AND DETECTION OF *PLASMODIUM* PARASITE BY QPCR

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Determining precise parasite quantification in real time PCR has been a challenging aspect in malaria diagnostics. In general, quantification of *Plasmodium* by qPCR is done by serially diluting a standard of a known parasite density were the source can be from a cultured parasites or clinical samples. The parasites density is determined by microscopy and described in parasite/µl which is known as a relative standard. A relative standard can lead to incorrect quantification because it may have difference on the source, parasite culture or clinical samples. It relies on microscopy being performed accurately and consistently. Absolute quantification is based on known concentration of DNA standard molecules such as plasmid DNA. We have developed an absolute quantitative multiplex qPCR for detection of *Plasmodium spp., P. falciparum* and *P.vivax* described in parasite/µl. Plasmids DNAs are constructed for qPCR assays by amplifying PCR fragments from genomic DNA from either clinical samples or cultures and cloned into TOPO TA vectors. The concentration of each plasmid DNA was determined in genomic equivalence (GE) and was used for subsequent experiments. All of the absolute qPCR assays performed with efficiency of more that 94%, R^2 values greater than 0.99 and the STDEV of each replicate was <0.167. Correlation of genomic equivalence to parasite/µl was established using standard clinical samples and or cultures. One copy of plasmid DNA was established to be equivalent to 0.12 parasite/µl for *Plasmodium spp.* assay, 0.54 parasites for *P. falciparum* assay and 0.16 parasite/µl for *P.vivax* assay. From this data, absolute qPCR can be expressed in parasite/µl. An absolute quantitative qPCR assay is better than a relative qPCR because it is more accurate and consistent. Plasmid DNAs are stable, can be easily produced in large quantities and stored for a long period of time. In addition, plasmid DNA production and quantification can be highly standardized ensuring more uniform quantification. Accurate quantification of parasites can have great impact on malaria diagnosis in clinical trials as confirmatory method to microscopy.

1309

DEVELOPMENT OF LOCAL EXPERTISE FOR PLACENTAL MALARIA HISTOPATHOLOGY IN TORORO, UGANDA: FROM COLLECTION TO INTERPRETATION

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Placental histology is a valuable technique to evaluate malaria epidemiology and control during pregnancy. Histology laboratories and expertise are usually confined to urban tertiary care hospitals and facilities are rare in Africa. The process of preparation requires specialized skills and training. As part of a study of malaria rapid diagnostic tests (RDTs) in pregnancy, placental specimens were collected in rural eastern Uganda (n=267) and southwestern Burkina Faso (n=548), areas of high and seasonal transmission, respectively. Specimens were fixed in 10% neutral buffered formalin and after 24 hours transferred to 70% ethanol for shipping and storage (4-8°C). On site in Uganda, paraffin blocks were

manually generated, and sections were Giemsa stained. Concurrently 30 biopsies per site, 30 paraffin blocks and stained slides were shipped to Seattle, USA, for external quality control (QC). Tissue was well preserved with no formalin pigment artifact. Blocks and stained slides were well prepared with minimal artifact. Processing and staining problems detected early were rapidly addressed. After one-on-one training on reference slides, study samples were interpreted by two trained technologists, with cross-checking against placental blood smears. A subset of all positive cases and 10% of negative cases were reviewed at University of Washington. The majority of supplies were locally available, however a microtome was imported by another research group at the same site, and microtome knives, charged slides and paraffin wax were imported. Challenges included exposure to alcohol, xylene, and formalin, and the physical distance to the nearest experienced histopathologist. The exercise demonstrated development of placental malaria histopathology expertise with robust QC in an inexpensive laboratory in a rural district hospital, showing successful implementation of capacity-building for a highly skill-dependent activity critical to study success, and providing potential for long-term reference-level histology for pregnancy studies in Africa.

1310

POINTING OUT MALARIA INFECTIONS WITH LASER POINTERS

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Detecting the presence of malaria parasites primarily relies upon; the highly accurate but time consuming method of PCR, the use of expensive and subjective RDT card tests, or the inexpensive but slow (up to 1 hr) microscopy-based methods which can yield false positives for 36% of samples and false negatives as high as 18% of the time. Inter-operator error in creation, staining, and visual analysis of the slides may contribute to this high error rate. Therefore, there is a need for novel malaria diagnostic techniques to identify which samples are potentially infected and help confirm negative diagnoses. Through a multidisciplinary effort we have designed an inexpensive, rapid malaria detection device (3 minutes) that detects the presence of hemozoin, a parasite byproduct of hemoglobin digestion. We place blood samples into the path of a polarized light beam in the presence and absence of a magnetic field. When the partially magnetic malaria hemozoin is present, it aligns with the magnetic field and acts as a reflector thus decreasing the amount of light reaching a light level detector on the far side of the sample. This decrease in light is directly proportional to parasitemia (R2=0.996) which can be detected at parasitemias as low as 0.00033% (17 parasites per microliter) which exceeds detection levels for microscopy without the need for staining or trained microscopists. Our long term goal is to translate this technology into a field ready, low-cost device, which can be used in malaria-endemic regions to enable rapid malaria diagnosis at the point-of-care.

1311

TRENDS IN PRESCRIBER ADHERENCE TO MALARIA TESTS IN HEALTH FACILITIES RECEIVING JOINT CLINICAL AND LABORATORY SUPERVISION VISITS

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During 2008-2012, the President's Malaria Initiative made considerable investments towards improving malaria diagnostics to promote the rational use of anti-malaria drugs in health facilities in sub-Saharan Africa. Through the Improving Malaria Diagnostics (IMaD) project, Ministries of Health in Benin, Ghana, Malawi, and Zambia, implemented quality assurance programs based on Outreach Training and Support Supervision (OTSS). In Ghana, laboratory supervisors implemented routine supervision and observed laboratory specific topics such as malaria microscopy (MM) and RDT performance. The same laboratory supervisors addressed prescriber compliance during their visit. In Benin, Malawi, and Zambia, supervision was implemented together by a laboratory and clinical supervisor. Laboratory supervisors focused on MM and RDT performance while clinical supervisors addressed fever diagnosis and prescriber adherence. Standardized checklists were used during each visit and improvements were tracked using a Microsoft Access database. In countries implementing joint supervision, general positive trends in prescriber adherence to microscopy and RDT results were observed: Benin 38% (MM) and 39% (RDT) percentage point improvement between visits 1-7, Malawi 17% (MM) between visits 1-3, and Zambia 23% (MM) and 21% (RDT) improvement between visits 1-4; In Ghana, where supervisory visits were implemented by laboratory supervisors only, no discernible trend was observed in prescriber adherence to negative tests: changes of 3% (MM) and -7% (RDT) were observed. Trends in prescriber adherence from OTSS data show that a joint approach to supervision had a greater impact on prescriber adherence to negative blood slide and RDT results than supervision conducted by laboratory supervisors alone, provider confidence improves when laboratory results are quality assured, and communication between the two cadres is strengthened. It should be noted that not all facilities have received a full cycle of visits due to the staggered nature of the roll-out of the OTSS program.

1312

CLINICAL SIGNS AND SYMPTOMS OF *PLASMODIUM FALCIPARUM* MALARIA INFECTION (PATENT AND SUB-PATENT) IN PREGNANT WOMEN LIVING IN AN AREA OF HIGH SEASONAL TRANSMISSION

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Malaria in pregnancy is a major public health concern in endemic countries. There a paucity of data on the association between peripheral parasitaemia and the presence of signs and symptoms of malaria during pregnancy. The objective of this study is to document the frequency of the attendance of pregnant women at health facilities with clinical complaints suggestive of malaria and to assess their parasitological status. To attend this objective, a hospital-based descriptive study at the maternity clinic was conducted in the rural district of Nanoro in Burkina Faso. A total of 600 pregnant women attending the antenatal care (ANC) were recruited, 200 pregnant women with signs and symptoms suggestive of malaria and 400 others without signs and symptoms were control group. The women were matched by gestational age and parity. For each woman, a capillary blood sample was taken for rapid diagnostic test, microscopy and hemoglobin test. A multivariate model was used to access each predictor of malaria. The overall prevalence of malaria was 42.6% (256/600) using the microscopy while anemia was found in 60.8% (365/600). Nearly a half (49.5%) of the women who displayed symptoms was parasitaemic and 39.5% of the asymptomatic women were parasitaemic. The most frequently encountered signs and symptoms were fever 36% (72/200), history of fever 29% (58/200) and headache 52% (104/200). The predictive positive values for fever were 53% (95%CI 41-64), history of fever 58% (95%CI 37-63) and headache 51% (95%CI 41-61). Signs and symptoms suggestive of malaria are quite frequent in pregnant women in intense transmission area. A large number of asymptomatic but parasitaemic women were found. For a better management of malaria in pregnancy, active case detection of all pregnant women attending the ANC should be performed to detect and treat earlier malaria infection.

1313

BLOOD SMEAR TEST FOR MALARIA CONFIRMATION AT THE COMMUNITY LEVEL: FEASIBILITY AND LESSONS LEARNED FROM SARAYA HEALTH DISTRICT, SENEGAL

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Following Rapid Diagnostic Test (RDT) and ACT introduction in 2009, health units were asked to confirm all malaria cases; this has been partially scaled up to the community level where Community Health Workers (CHWs) were trained to use RDT. Smear blood tests were realized only in the laboratories with laboratory technicians. In Saraya district thick and thin blood smears were introduced to confirm all malaria cases in 24 villages involved in a Seasonal Malaria Chemoprevention research project. The objective of the study was to assess the feasibility of smear blood tests at the community level. Saraya district is located in South East Senegal, bordering Mali and Guinea Republic. Health staff was very limited, and with a strong network of community health workers and malaria village volunteers called DSDOM. Twenty four CHWs and malaria volunteers were trained by staff from the medical school, parasitology laboratory for 3 days to perform Blood smears with practical sessions. They were asked to complete RDT, thick and thin blood smears for all patients under 10 with fever. Blood smear tests were kept in a box and collected by supervisors. Close follow up were made by supervisors, mainly in the first month for continued training and improvements. Slides were read at the Medical school, parasitology laboratory. CHW performed 1635 blood smear tests between July and November 2011; 68.47% were positive, 31.47% negative, 0.06% not readable. Parasite density mean was 22.8 [13, 521] and nearly all malaria cases were due to *Plasmodium falciparum* (98%) with only 2% of malaria due to *P. malariae* and *P. ovale*. Blood smears can be performed at the community level by lower educated personnel with formal training and close follow up; this would be helpful to be more accurate on malaria diagnosis and non malaria febrile illnesses as well in remotest under served areas.

1314

A REVIEW OF MALARIA RAPID DIAGNOSTIC TESTS (RDT) GUIDELINE IMPLEMENTATION IN A DISTRICT HOSPITAL IN GHANA: HAS RAPID TESTING BEEN PRIORITIZED?

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Rapid diagnostic tests (RDTs) can improve timeliness and accuracy of malaria diagnosis. This can help to slow down the development of antimalarial drug resistance by promoting appropriate treatment. Since 2009, revised malaria management policies in Ghana promote testing by microscopy or RDTs, before treating all suspected malaria cases aged five years and above. In this study we reviewed the use of RDTs in a district hospital over a two-year period following the implementation of 'test-before-treat' policies for malaria in Ghana. A random sample of 500 malaria cases recorded at the Nkawie-Toase District Hospital from January 2010 to December 2011 were identified and reviewed**.** For reference visits where the clinician made a differential diagnosis of malaria, only 3.6% (95%CI: 2.2-5.7) of reviewed cases were tested with RDTs compared to 13% (95%CI:10.2-16.3) by microscopy. For cases with repeat visits within 6 months of the reference attendance, percentage RDT-use decreased to 0.6% (95%CI: 0.01 - 3.5), while testing by microscopy increased to 26.1% (95%CI:19.3-33.8). RDT use ranged from 1.6% to about 4% (p=0.09), from low to high malaria incidence months. Testing with microscopy appeared strongly associated with seasonality of malaria, almost doubling from 10% in non-peak, to 19.3% (p=0.005) in peak malaria incidence months. Testing by microscopy was more frequent than RDT use during the period under review. These findings suggest that rapid malaria testing was poorly implemented in this district hospital over the study period, despite existing policy revisions in Ghana. Investigating RDT utilization in similar referral level facilities is essential to understand and to improve the implementation of current malaria testing guidelines in Ghana. This information will be useful to advise investments in rapid diagnostics for malaria, and to improve their application in limited resource settings.

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RAPID DIAGNOSTIC TEST (RDT) PERFORMANCE OF THE MALARIA GOLD MINING PROGRAM IN SURINAME: A COMPARISON BETWEEN RDT AND BLOOD SMEAR RESULTS

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Currently malaria infections occur mainly among persons (ca. 15,000) engaged in small-scale gold mining and related activities. The mining areas are remote from the existing healthcare services. To address this problem, a system of quick diagnosis and treatment was established by training lay persons (e.g shopkeepers) in gold mining areas to perform malaria diagnosis and to treat uncomplicated malaria. They are called Malaria Service Deliverers (MSD). Also in the city, in the gold miners' neighborhood, the Tourtonne laboratory was established to provide similar services. For each RDT performed a blood smear is taken and examined by Tourtonne Laboratory (TL) for quality control of the RDT. Good RDT performance is the cornerstone of the MSD system. The RDT results from 2007 through the first quarter of 2012 were compared with the Blood Smear results. 4489 slides received from the MSD were readable for comparison. The overall sensitivity was 83.3% (81.2 - 85.2%), and the overall specificity 91.8% (90.8 - 92.7%); a PPV of 80.8% was calculated. For the TL 6761 RDT results were available for comparison. The sensitivity was 81.3% (79.7 - 82.8%), the specificity was 96.2% (95.6 - 96.7%) and the PPV was 92.7%. Looking specifically at the performance of RDT related to *Plasmodium falciparum,* the sensitivity, specificity and PPV were respectively 83.1%, 94.2% and 62.6% for MSD versus 84.8%, 95.6% and 82.7% for Tourtonne laboratory. The sensitivity of both the MSD system and TL were lower than the expected sensitivity (95.3%) calculated by the manufacturer. The specificity of both on the other hand was according to expectations. As the accurate diagnosis and treatment for especially *falciparum* malaria is of paramount importance, false negative tests should be avoided. Further research e.g. parasitaemie level, storing conditions, reader variability is needed to explain the difference found between the expected and found sensitivity.

1316

INVESTIGATING THE OPTIMAL SAMPLING SCHEME FOR MEASURING PARASITE CLEARANCE WITH THE PARASITE CLEARANCE ESTIMATOR

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The emergence of artemisinin resistance in South East Asia threatens the efficacy of artemisinin derivatives (AD). Since the pharmacodynamic hallmark of AD is rapid parasite clearance, the clinical phenotype of slow clearance characterises resistance. Frequent parasite counts are needed to define clearance rate but it is uncertain what sampling frequency is required to ensure reliable estimates. We selected 2841 parasitaemia-time profiles from clinical studies in which 6-hourly parasite counts were available in the first 48 hours (h). Patients were treated with an artesunate alone or in combination with a partner drug. WWARN's Parasite Clearance Estimator estimated the median (range) parasite half-life (HL) as 3.2 (0.7 - 17.5) h. Four measurement schedules (at 0,6,12,24 or 0,6,18,24 or 0,12,18,24 or 0,12,24 h and then every 12h) were investigated. The median (range) for the difference between the original HL estimate and that from the 4 schemes were -0.02 (-3.4 to 3.8), -0.06 (-3.3 to 3.5), -0.09 (-3.6 to 3.4), -0.15 (-5.0 to 3.6) h, respectively. The overestimation of the HL by the restricted schemes was greater for profiles with short reference HL. Bootstrapping was then used to estimate the sampling distribution of HLs for two subsets of the population with: (A) fast clearance (20% of reference HL>3h) and (B) slow clearance (50% of reference HL>3h). In both subsets, the median HL was overestimated by the 4 schemes (A:91 -100%, B: 79-97% of bootstrap samples), but by ≤0.5h for nearly all samples. The schemes overestimated the proportion (%) of profiles with HL >3h, on average by 39, 44, 54, 72% (A) and 6, 7, 9, 12% (B), relative to the scheme with 6 hourly measurements, respectively. The proportion of profiles with HL longer than 6h in bootstrap samples was very similar for all restricted schemes. Our data indicate that HL can be best estimated by including samples at 6 and 12h while every 12h counting is satisfactory in patients with slow clearance.

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WORLDWIDE ANTIMALARIAL RESISTANCE NETWORK (WWARN) TOOLKIT: PROMOTING HARMONIZATION OF ANTIMALARIAL RESISTANCE EPIDEMIOLOGICAL OUTPUTS

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To meet the known threat of parasite resistance to artemisinin-based therapies, the WHO's Global Plan for Artemisinin Resistance Containment stresses the need for more quality-assured antimalarial efficacy data. The WorldWide Antimalarial Resistance Network (WWARN) response is an online Toolkit that guides research scientists to collect the reliable, comprehensive evidence needed by the public health community to identify and contain antimalarial drug resistance. The Toolkit contains a growing portfolio of essential tools and services to promote high-quality antimalarial efficacy and laboratory testing. These include guidance on study protocol design, tools for data collection and analysis and technical procedures, supported by proficiency testing and reference material programmes, training workshops and online courses. The Toolkit will assist researchers - particularly those working in resource-limited environments - in the design, conduct and interpretation of their studies, thereby facilitating high-quality prospective data collection, and reducing data heterogeneity. The standardised Toolkit data outputs from *in vivo* studies and laboratory tests can be pooled - across studies, time, and place - uncovering subtle trends or sub-population effects with higher statistical certainty. Increasing the ease and potential for data mining in turn allows complex issues, like antimalarial resistance, to be understood more quickly and cost-effectively and with less duplication of effort. We will describe the components of the Toolkit and present the 'roadmap' that guides scientists progressively through the steps to plan and run antimalarial resistance research projects and how to use the various tools and services.

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LONGITUDINAL STUDY OF SULFADOXINE-PYRIMETHAMINE (SP) RESISTANCE IN TURBO, COLOMBIA

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Pyrimethamine was introduced in the 1950s in South America as a mass treatment in Venezuela. By 1968, pyrimethamine-resistant parasites were found in Colombia and resistance rapidly disseminated in the Amazon and Orinoco basins. However, SP resistance in Colombia is unevenly distributed, showing high resistance in the Amazon basin to moderate levels in the Caribbean, the Cauca Valley and northwestern regions. In this study we characterized a total of 145 *Plasmodium falciparum* samples from Turbo, a port town in Antioquia Department, collected during years 2002 to 2009 and characterized point mutations in two genes that have been implicated in resistance to SP, dihydrofolate reductase (dhfr) and dihydropteroate synthase (dhps). The treatment given to the patients in this area during 2002 until 2006 was a combination therapy of amodiaquine and SP, which was changed to artesunate and mefloquine in 2007 and then to Coartem in 2008. We found that pyrimethamine-resistant double mutants (50C/51I/59C/108N/164I) are nearly fixed in the population, while both sensitive and resistant sulfadoxine genotypes (436S/437G/540K/581A/613A) were present in the population. We also assayed neutral microsatellite markers around the dhfr (chromosome 4) and dhps (chromosome 8) loci to get an idea of the strength of selection. According to the microsatellite haplotypes for the dhfr and dhps SP-resistant alleles, the dhfr double and dhps single mutants seem to have a single origin. Further studies are required to determine the increased in frequency of SP sensitive parasites, as well as to characterize the gene flow between the southwestern populations, where SP is still efficacious, and the northwestern populations of Colombia where moderate resistance has been documented.

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WORLDWIDE ANTIMALARIAL RESISTANCE NETWORK (WWARN) *IN VITRO* PROFICIENCY PILOT PROJECT: DETERMINATION OF THE INTERLABORATORY VARIABILITY OF IC_{50} ESTIMATES IN *PLASMODIUM FALCIPARUM* REFERENCE CLONES

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In vitro testing is a key component of resistance surveillance as drug susceptibility can be tested without the influence of human confounders, such as immunity and pharmacokinetic parameters. Furthermore the

effect of single compounds in a combination therapy can be evaluated. Currently a wide range of methodologies and conditions are being used to perform drug susceptibility testing in the global community of *in vitro* laboratories. Although there is no gold standard *in vitro* protocol that is suitable for all drugs in all different settings, several aspects of *in vitro* methodology can be standardised to reduce variability. In this study we assessed whether the inter-laboratory variability of drug susceptibility testing could be minimized by introducing simple standardisation measures. Fifteen participating laboratories used their established methodology to test the drug susceptibility of *Plasmodium falciparum* reference clones 3D7 and W2 on several occasions. WWARN provided the following measures to improve standardisation: 1) genetic validation of reference clones by microsatellites and *pfmdr1* gene copy number at the start and close of the pilot project; 2) validated test drugs – chloroquine, mefloquine, desethylamodiaquine and dihydroartemisinin – supplied from the WWARN Reference Material Programme; 3) standardised data analysis using the WWARN *In Vitro* Analysis and Reporting Tool (IVART). Comparing data from different laboratories improves understanding of the range of variability encountered with different *in vitro* readout methods when other parameters have been standardized. These results will be presented and used to design a Proficiency Testing programme to improve standardisation of *in vitro* assessment across the malaria community.

1320

GENOMIC APPROACH FOR TARGET IDENTIFICATION OF ANTIMALARIAL CYCLOPROPYL CARBOXAMIDES

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One of the current antimalarial drug discovery approaches is focused on whole cell screening. One of the greatest challenges working with compounds identified in phenotypic screenings is the complete lack of knowledge of the molecular target responsible for antimalarial activity. Cyclopropyl carboxamides (CCAX), a chemical class not described previously as antimalarial drugs, have been identified recently from a whole-cell screening as potent inhibitors of *Plasmodium falciparum* drug-sensitive and resistant strains, as reported previously. Moreover, this series shows a promising *in vivo* oral efficacy in *P. falciparum* mouse models. This might indicate an antimalarial mode of action different from already known resistant mechanisms, although only the identification of the target responsible for the antimalarial activity could confirm it. Despite a potent *in vitro* activity, further characterization of the molecules has revealed an unusual propensity to develop resistance. The frequency of spontaneous resistance is one order of magnitude higher than in the case of atovaquone when using W2 strain. To investigate the resistance mechanisms of this series and to achieve the identification of the cyclopropyl carboxamide antimalarial target we have selected seven independent pure clones that have been extensively characterized. Despite of the high level of resistance (two orders of magnitude) none of them shows sensitive differences in terms of growth rate compared with the parental strain. We have purified genomic DNA of the different clones and started a full genome sequencing approach in order to identify the determinants responsible of CCAX resistance. The identification of this target would help to the progression of this chemical series and to a better understanding of antimalarial resistance.

1321

PHARMACODYNAMICS OF ARTEMISININ-BASED COMBINATION THERAPIES (ACTS) IN A RODENT MODEL OF ARTEMISININ-RESISTANT MALARIA

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The emergence of the delayed parasite clearance phenotype in *Plasmodium falciparum* parasites present in the Greater Mekong subregion, as reported previously, highlights the urgent need to identify antimalarial therapies and regimens that can adequately treat these infections. In this study, we have employed an animal model to monitor the course of rodent malaria infection after treatment with Artemisinin-based Combination Therapies (ACTs). In addition to providing insight into the pharmacodynamic properties of existing ACTs when used against artemisinin-resistant strains, our work shows that the artemisinin-resistant *Plasmodium berghei* SANA strain can be treated successfully with an artesunate-pyronaridine combination therapy at similar drug concentrations that are curative for infections with the parental drug-sensitive N strain. The 30-day outcomes indicate that SANA resistance to artemisinin can be overcome with the combination of pyronaridine-artesunate. Piperaquine, which is currently used in combination with dihydroartemisinin in Southeast Asia, also proved to be effective in clearing parasite infections after three doses in the animal model. Of the five partner drugs tested, pyronaridine was the most effective at suppressing the recrudescence of SANA parasites.

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EFFICACY AND EFFECTIVENESS OF ARTEMETHER-LUMEFANTRINE AFTER FIVE YEARS OF WIDE SCALE USE IN TANZANIA

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Artemether-lumefantrine (AL) is the most widely adopted ACT in sub-Saharan Africa. The recent emergency of artemisinin resistance in *Plasmodium falciparum* malaria in South East Asia, characterized phenotypically by slow parasite clearance following ACT treatment, highlights the need for both detailed follow up monitoring of efficacy/effectiveness of AL, including detailed assessment of parasite clearance time, and surveillance . identification of molecular markers of resistance to AL as an early warning system. This study is conducted in two rural sites in Bagamoyo and Kibaha Districts, We are conducting a randomised clinical trial to assess the efficacy and effectiveness of AL in children with uncomplicated malaria, including parasite clearance time, selection of molecular markers of resistance, identify factors associated with poor adherence after five years of wide scale use in Tanzania. We are enrolling patients 6 months-10 years with confirmed malaria by finding parasites in blood samples. Patients are randomly allocated to either supervised (admitted to the health facility for 3 days) or unsupervised (at home) artemether-lumefantrine (Coartem®) treatment, and then they are reviewed every week for 42 days, to monitor treatment outcomes. Study nurses make home visits to assess treatment adherence through parent/caretaker interview and blister pack pill count. Standardized procedures recommended by WHO are used to accurately detect and document drug resistant malaria and lumefantrine drug levels on day 7. Data collecting will be completed August 2012.

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PLASMODIUM FALCIPARUM IN VIVO EARLY RESPONSE TO ARTEMETHER-LUMEFANTRINE THERAPY IS ASSOCIATED WITH ABC TRANSPORTER TRANSCRIPTS

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Increased expression of ABC transporters has been associated with decreased clinical drug response in several different pathologies. *Plasmodium falciparum* malaria is no exception and increased copy number of the parasite P-glycoprotein homologue has been associated with resistance against several antimalarials, including lumefantrine and artemisinins. The aim of this work was to investigate the expression of *P. falciparum* ABC transporters in a clinical setting, upon treatment with artemether-lumefantrine (AL), the most used treatment against *P. falciparum* malaria. The clinical trial was conducted at Fukayosi Primary Health Care Centre, Bagamoyo District, Tanzania in 2006. A total of 50 patients: age 1-10 years were included, hospitalized and treated with standard 6 doses of AL. Venous blood samples were taken at 0,2,4,8,16,24,36,48,60,72 hour and preserved for nucleic acid extraction.. RNA was extracted and quality controlled at Karolinska Institutet. For the time points up to 24h cDNA was synthesised and analysed by Real-time PCR for relative quantification of pfmdr1, pfcrt, pfmrp1, pfmrp2 using the endogenous control seryl-tRNA synthetase (PF07_0073). Gene expression relative to the control was calculated using the ΔΔCt method. After initiation of AL treatment the expression of pfmrp1 increased significantly whereas the expression of pfmdr1, pfcrt and pfmrp2 significantly decreased. Our results emphasises the likely importance of pfmrp1 in artemisinin combination therapy drug resistance.

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EVALUATING THE ROBUSTNESS OF PARASITE CLEARANCE RATE MEASURES USING HERITABILITY

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Measurement of malaria parasite clearance rates following artemisinin treatment requires sequential parasite counts taken at intervals following treatment. Frequent sampling is ideal, but extremely labor intensive, and the optimal strategy for obtaining robust clearance rate estimates while minimizing sampling effort is poorly understood. We evaluate a variety of metrics (24 and 48hr parasite reduction ratios, time to parasite clearance, clearance half-lives ($t_{1/2}$) measured using 6-24 hourly sampling until clearance or for the first 48 hrs only). We also evaluate the effect of different slope fitting procedures (ignoring or incorporating time lags) on the robustness of clearance estimates. We perform these analyses using parasite clearance data collected from 1731 hyperparasitemic patients from a region of emerging resistance on the Thai-Burma border. All parasites were genotyped using 96 single nucleotide polymorphisms, and we used heritability (the proportion of variation attributable to parasite genetics) to evaluate the robustness of each measure. Our assumption is that the most robust measure will show the highest heritability, while less useful measures will show lower heritability due to measurement error. The results of these analyses will be presented.

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SUPPRESSING ANTIMALARIAL DRUG RESISTANCE WITH COMPLEMENTARY INHIBITORS: CATCHING *PLASMODIUM FALCIPARUM* BETWEEN A ROCK AND A HARD PLACE

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Managing drug resistance is a core problem in anti-malarial drug therapy. Resistance has arisen to all drugs in clinical use. Combination therapy is a key tool for delaying the development and spread of resistant parasites. Most combinations use two drugs with different mechanisms of action. We explore here the possibility of using two agents acting on the same target - one drug that selectively inhibits the wild-type target, and a complementary partner drug that selectively inhibits the most likely drug-resistant mutations. Selection of malaria parasites resistant *in vitro* to either alkylthiophene- or triazolopyrimidine-based Dihydroorotate Dehydrogenase (DHODH) inhibitors resulted in mutations in the drug-binding pocket of DHODH, which had been previously determined through crystallography and biochemical characterization of purified protein. Using those mutants resistant to both the alkylthiophene and triazolopyrimidine inhibitors we re-screened a library of DHODH inhibitors. This screen yielded several compounds 10-100-fold more potent against the mutants than the wild-type parasites. Subsequent selection of resistance to these selectively potent compounds in the alkylthiophene-resistant strains resulted in reversion of the DHODH gene back to wild-type, confirming the key role of the mutation. Modeling and molecular dynamics simulations are being used to probe the mechanisms of heightened sensitivity to different compounds. Combination therapy that exploits target reciprocity traps malaria: escape from the primary drug results in increased sensitivity to the secondary drug. Selective pressure on the partner compound is predicted to be reduced; it acts only against the small population of parasites that become resistant to the primary compound. In targets that tolerate few mutations, the fitness costs of becoming resistant to both complementary inhibitors may provide a path for suppressing drug resistance.

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EMERGING COARTEM RESISTANCE ASSESSED BY DAY THREE PARASITEMIA IN SURINAME

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In 2004 Suriname changed its first line treatment for *Plasmodium falciparum* malaria to an artemisinin based combination therapy (ACT), introducing Coartem.. This and other measures resulted in a more than 90 percent decrease of malaria. Currently malaria cases are mainly seen in gold miners in the interior. In this population adherence to treatment is poor and also the use of counterfeit medication is widespread. Following WHO recommendations, the efficacy of the treatment was evaluated in 2006 and found to be adequate. A study was undertaken to assess Coartem efficacy in patients with *P. falciparum* malaria. Consenting patients with *P. falciparum* malaria mono-infection were enrolled and followed to assess the course of clinical symptoms and parasitemia. Because of the current low number of cases available for a 28 days follow up, in this assessment we also included day 3 parasitaemia, as a clinical endpoint The treatment was directly observed; patients were followed until parasite clearance plus one day and then on day 7, 14, 21 and 28. 67 Patients were enrolled, of whom 9 were withdrawn because of protocol violations. There were no reports of serious side effects. From the

remaining 58 patients, 5 were lost to follow up before parasite clearance. Only 11 patients were followed for the full 28 days period, none of whom had recurrent parasitaemia.. From the 53 patients that were followed at least until parasite clearance, 15 (28.3%) had still parasites on day 3. From 11 patients that were followed until day 28 only 1 had a positive slide on day 3, which became negative on day 4. Comparing these results to those of 2006 we found that at that time the incidence of day 3 parasitaemia was 1.6 percent, with 95% of cases with a negative slide on day 28. We conclude that the rate of day 3 parasitaemia has significantly increased in 2011 ($p < 0.001$). This may be an indication for emerging resistance to Coartem. It is suspected that this may be due to the (improper) use of counterfeit ACT.

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MALARIA AS A CAUSE OF ACUTE FEBRILE ILLNESS IN AN URBAN PEDIATRIC POPULATION IN GHANA

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Annually about 500 million people become severely ill with malaria. Over 90% of cases and deaths occur in Sub-Saharan Africa with children under five years and pregnant women being affected mostly. In Ghana, about 3.7 million cases of malaria were reported in 2010 out of which only 26% were confirmed by laboratory testing. Most febrile cases are treated as malaria sometimes with fatal consequences though they may not be malaria. This could explain the high proportion of funds that the Ghana Health Insurance Authority spends on the treatment of malaria. This study sought to determine the proportion of acute febrile illness in children under five years due to malaria. A hospital based surveillance system recruited children less than five years who reported at the out-patient department of an urban hospital with fever ≥ 37.5°C at the time of visit from February 2009 to February 2010. Parents/guardians who consented were interviewed using a structured questionnaire and the child examined by a clinician. Capillary blood through a finger prick was used for a thick blood film using Giemsa and viewed under the microscope for malaria parasites. Out of the 605 children with fever whose blood samples were taken for microscopy, only 68 were positive for malaria, giving an overall positivity rate of 11.2%. Malaria was equally distributed among males and females, the proportion malaria cases increased as age increased. Of the 492 children whose reports were available, 80% of the children were diagnosed by clinicians as having malaria either alone or in combination with other diseases and were treated with anti-malarials. The treatment of febrile cases based solely on clinical symptoms has been shown to be less cost effective than confirming the diagnosis with a laboratory test and also promotes the occurrence of drug resistance.. Clinicians should look out for other causes of fever rather than treating almost all febrile cases as malaria. The National Malaria Control Programme has intensified efforts to increase laboratory testing before treatment.

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A COMPREHENSIVE RISK MAP FOR MALARIA IN KINSHASA, DEMOCRATIC REPUBLIC OF CONGO

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The Democratic Republic of Congo (DRC) is the second most malarious country in the world. However, there is a paucity of epidemiological data on the risk pattern of malaria. In 2009 (dry season) and 2011 (end of the rainy season) two two-stage cluster sampling malaria surveys were conducted in the capital city Kinshasa with the twofold aim of (1) assessing malaria parasite prevalence, anemia and associated malaria risk factors, and (2) producing a malaria risk map using a geographic information system (GIS). A total of 6410 children aged 6-59 months (3058 in 2009 and 3352 in 2011) were tested for both malaria (using rapid diagnostic tests) and anemia (by HemocueTM). Nine health zones (HZ) were sampled in 2009 with an average prevalence for malaria and anemia of 6.6% (95% CI 5.8-7.5) and 66.0% (64.5-67.4) respectively, while in the 25 HZs in 2011 the prevalence was 17.0% (15.7-18.3) and 64.2% (62.6-65.9). Overall, the prevalence rate for both surveys was 11.9% (11.2-12.8) for malaria and 65.1% (63.9-66.7) for anemia. To ensure comparability of the results between surveys, two HZs from 2009 were resampled in 2011. Prevalence for malaria in 2009 and 2011 was: Ngiri Ngiri 1.0% versus 0.8% and Selembao: 14.1% versus 26.8%. Prevalence for anemia was: Ngiri Ngiri 62.5% versus 55.4% and Selembao: 67.1% versus 61.4%. In a multivariate analysis of the 2011 data significant protective factors for malaria risk were: educational level of the respondent (OR = 0.12, 95% CI: 0.03 - 0.56) and sleeping under an ITN (OR = 0.52, 95% CI: 0.43 - 0.63). All key parameters were mapped to the level of the HZs (n=35). Malaria parasitemia, anemia and fever prevalence were found to be much lower in the city center than in the peri-urban suburbs, where transmission rates remain high. ITN usage showed the opposite pattern. These maps provide for the first time a comprehensive picture of the epidemiology of malaria in Kinshasa and provide solid baseline information for planning future malaria control interventions.

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LINKING THE INCIDENCE AND AGE PATTERNS OF CLINICAL MALARIA TO PARASITE PREVALENCE USING A MATHEMATICAL MODEL

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Estimating the changing burden of malaria disease remains difficult due to limitations in health reporting systems in those countries with the largest burden of disease. Methods extrapolating from parasite prevalence data are therefore often employed. We present an approach to estimating disease incidence from prevalence data accounting for the changing age distribution of cases that occurs as transmission declines. We use a transmission model to capture the shifting age-pattern of disease at different transmission intensities through dynamically modelling the acquisition and loss of immunity. The model is fitted to age-stratified data on the incidence of uncomplicated malaria due to *Plasmodium falciparum* from 24 sites in 9 sub-Saharan African countries. We used Bayesian methods, and accounted for variation in treatment rates and reporting methods (active versus passive case detection). We estimate that passive case detection picks up 32% (95% credible interval: 18-56%) as many cases as daily active detection, and weekly detection 77% as many (95% CrI: 63-88%). However, there was wide variation in incidence between studies that cannot be explained by differences in case-finding or case definitions such as parasitaemia thresholds, and so substantial uncertainty remains in the incidence at any given transmission intensity. We estimate that at a parasite prevalence in 2 to 10 year-olds of 60%, 50% of cases occur in under-fives and 10% in over 15s; at a prevalence of 20%, 21% are in under-fives and 38% are in over 15s; and at a prevalence of 5%, 11% are in under-fives and 59% in over 15s. As our transmission model includes the principal control measures, these results will allow us to predict the impact of interventions on the incidence of clinical malaria.

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RATIONALE AND DESIGN OF CCM IN SARAYA DISTRICT: RESULTS AND IMPLICATIONS FOR POLICY IN RURAL SENEGAL

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Following the Abuja conference, malaria control strategies improved malaria patterns in Senegal with 3% of prevalence in outpatients in 2009. Figures hid disparities between Northern regions where morbidity was low and the southeast with high malaria incidence. Furthermore the data came only from health units only. To fill the gap in most under served areas, Community Case Management (CCM) was scaled up in rural Senegal with Community Health workers (CHW) and village volunteers called DSDOM. The objective of the study was to evaluate CCM at larger scale and identify policy implications for malaria. The project was held in Saraya district located in south east Senegal. It covers 6837 km2 for 102 villages with an estimated 40000 population; 70% lived at more than 15 km of the nearest health unit. CCM was done in 47villages with CHW or village volunteers, trained in malaria diagnosis and treatment. A Community Households cluster survey with Knowledge, Attitude, Practices (KAP) and Treatment seeking behavior were completed, quality of data assessed and traditional healers involved from June 2010 to December 2011. 683 heads of household were interviewed. Mosquito as the malaria vector was recognized by 81% of household heads, 93% cited mosquito nets as protective, RDT was well known (71%) and ACT as well (62%); 33% of respondents knew of potential adverse events, and use of LLINs the night before the survey was reported by 80% of respondents. In 73% of households 1 member was ill during the last 15 days, 91% had fever, and first visits were done by CHWs (34%), nurses (17%), DSDOM (15%) and traditional healers (7%); 44% of patients completed consultations within 24 hours. ACT was administered to 55% of patients. Few patients (N=52) sought second treatment mainly from CHW (36%) and traditional healers (18%). 15.491 visits were documented. 80% were reported by CHWs, about 50% were children under 10 years; 11.479 RDT were completed and 74% were positive; referrals (2005) were made and 6 deaths recorded. 36 traditional healers were visited by 67 patients. They referred 65 patients, 63% were RDT positive. The study revealed the central place of CHWs and the need to re-evaluate policy in under served areas, especially CHW status, the training curriculum and discussions on incentives to sustain filling the gap.

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IMPACT OF *PLASMODIUM FALCIPARUM* INFECTION ON HEMATOLOGICAL PARAMETERS IN CHILDREN WITH ABNORMAL HEMOGLOBIN LIVING IN A MALARIA ENDEMIC AREA OF BURKINA FASO

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Malaria is the most common cause of childhood morbidity in Africa, having varied haematological consequences. The high prevalence of HaemoglobinS/C is associated with the protection against malaria during childhood. Much less is known about the effect of HbS and HbC on malaria infection and haematological parameters. Susceptibility of the human host to malaria infection and haematological parameters has been reported to be influenced by some genetic factors as abnormal haemoglobin.The aim of this study was to evaluate haematological parameters in children less than fifteen years of age with abnormal haemoglobin genotypes and malaria infection. The study was conducted in 2008 in rural villages. It consisted of a combination of 2 cross-sectional surveys during the low and high malaria transmission. During each survey, each child was clinically examined, and thick and thin blood films were prepared for malaria diagnosis. The full blood count was performed with a haematology analyzer and an additional blood specimen was taken to determine the haemoglobin genotypes by PCR. In total, 406 children were recruited, 176 and 230 during the high and low seasons, respectively. Prevalence of Hb genotypes during the high and low season was: normal haemoglobin AA (76.7 and 65.7%) and abnormal haemoglobin (22.2 and 25.3%). There was no difference between the two groups in terms of leucocyte count and haemogloblin level if the subject was infected or not. However, during the low season, abnormal haemoglobin children without parasitemia tended to have higher lymphocyte counts (p=0.02), monocyte counts (p=0.02), red blood cell counts (p=0.03) and neutrophil counts (p=0.01), as compared to normal haemoglobin group. The platelet counts differed between the two groups for healthy children during the high season (p=0.002).The comparison of haematology parameters within haemoglobin type showed that basophil, lymphocyte and monocyte counts were significantly lower during high season. Basophil, eosiniphil, red blood cells, haematocrit, haemoglobin and monocyte counts in the malaria-infected normal haemoglobin group were significantly lower. In conclusion, these findings suggest that malaria parasites may affect the haematopoiesis of children living in malaria endemic area. Genetic factors, such as abnormal heamoglobin genotype, also influenced haematological parameters if subjects were not infected.

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ANTHROPOMETRIC GROWTH TRENDS DETERMINED BY WHO (2006) AND CDC (2000) GROWTH CRITERIA FOR CLINICALLY WELL INFANTS ENROLLED INTO A TRIAL OF SINGLE DOSE FANSIDAR FOR PRESUMPTIVE TREATMENT OF MALARIA IN RURAL NORTHEASTERN GHANA

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Current benefits of Fansidar-based intermittent preventive treatment for malaria during pregnancy (IPTp), and infancy (IPTi), prompted us to look retrospectively for evidence of a positive Fansidar effect on growth in clinically well young children that were randomized into a placebo-controlled, single-dose Fansidar trial at the peak of malaria season in rural Ghana. Growth charts from CDC (2000) and the newer WHO (2006) growth standards were used to determine within- and between-group differences at treatment baseline (Aug. 2001) and follow-up endpoint (Jan. 2002) among girls (n = 261) and boys (n = 237) enrolled in that four month trial. Weight-for-Age Z (WAZ) and Weight-for-Height Z (WHZ) scores derived from CDC growth charts were significantly lower (worse) than those derived from the WHO growth standards, while Height-for-Age (HAZ) Z scores were significantly higher by the CDC scale. Consequently, frequencies of underweight and wasting based on the CDC growth curve were greatly inflated over those derived from WHO standards and estimates of stunting were much lower by the CDC scale. Surprisingly, baseline and endpoint comparisons between sexes for mean WAZ, WHZ, and HAZ scores by both CDC and WHO growth criteria revealed better, more normative growth for girls-significantly improved over boys for all

three indices according to WHO criteria whereas by CDC criteria only HAZ was significantly improved. Endpoint comparison based on the newer WHO growth standards found no change in either sex for WAZ and % underweight, but WHZ and % wasting were improved significantly in boys. In boys and girls HAZ and % stunting worsened significantly; a result reflected by the two growth scales but which disappeared when sexes were combined. Analyses that combined sexes found no significant differences in growth indices that were associated with Fansidar or bednet effect, but comparison with a non-enrolled age-, sex-, and location-matched control revealed highly improved growth indices, by both CDC and WHO standards, for the cohort that had been subjects during this brief study.

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COMMUNITY HEALTH WORKERS AS AN EFFECTIVE CHANNEL FOR DELIVERY OF CHILD HEALTH INTERVENTIONS: EXPANDING THE KNOWLEDGE BASE

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A systematic literature review was conducted to assess the published and unpublished evidence on the effectiveness of strategies to improve community case management (CCM) of malaria. Specific objectives were to investigate interventions to (i) increase the coverage and quality of services provided by community health workers (CHWs) responsible for malaria case management; (ii) strengthen referrals from community to facility-based providers; (iii) increase the capacity of health systems (HS) to support CCM; and (iv) integrate malaria diagnosis and case management with other health services at the community level. Thirty-six studies were included in the review, the majority (32) reporting reasonably standard indicators of CHW performance. Findings show that CHWs are able to provide good quality of care, including performing simple procedures such as rapid diagnostic tests. Appropriate training and regular supportive supervision are important facilitating factors. However, crucial to the sustainable success of CHW programmes is the strengthening of HS capacity to support commodity supply, supervision, and appropriate treatment of referred cases. The little evidence available on referral systems from the community to health facility level suggests that this is a priority area that needs attention. There are few published studies on integrated CCM, although this is now the direction that policy and programmes are moving. Adding additional tasks does not reduce the quality of malaria CCM, provided sufficient training, supervision and support is maintained. However, with the exception of pneumonia treatment, reporting on the quality of delivery of additional interventions is limited. Amongst included studies, 11 reported on referral, 11 on HS capacity and 9 on iCCM; however not all data was quantitative and indicator definitions varied, making direct comparisons challenging. There is a need to encourage implementers to evaluate programmes robustly using standardised indicators and share their findings with other programmes to enable broader lesson learning.

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RISK FACTORS ASSOCIATED WITH MULTIPLE MALARIA INFECTIONS IN BANGLADESH

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Malaria is endemic in Bangladesh. In 2011, there were 51,773 episodes and 36 deaths attributed to malaria with most cases occurring in the Chittagong Hill Tracts (CHT), Bangladesh. This study identifies environmental and socioeconomic malaria risk factors and determines the spatial distribution of malaria in an endemic area in CHT. Longitudinal data on malaria incidence were collected from 1634 households in 54 villages (total population 7922) from January 2009 to December 2010. Hydrological, topographic, climatic and socioeconomic risk factors were used as potential predictors for malaria infection. Spatial malaria patterns were observed. Relative risk ratios were calculated to identify possible reasons for zero, one and >1 malaria infections in the study population. There were 509 malaria cases (6.4%) during the study period. These were distributed heterogeneously between villages. Children were most vulnerable to malaria infection. About 21.8% of homesteads accounted for all the malaria cases in the study area. The multivariate analysis with socioeconomic risk factors showed that bed net ratio (number of nets per person per household), ethnicity, house wall construction material, and household density had significant relationships with malaria incidence. Among the topographic and hydrological risk factors, households within two kilometers of a 4th order streams were at highest risk of malaria infection. In multinomial analysis belonging to the Bengali ethnic group, house walls made of mud and high household density were associated with a high risk for multiple malaria infections. High bed net ratio, belonging to the Tripura ethnic group, household heads having a non-specific ('other') occupation were associated with a low risk for multiple malaria infections. No clear relationship was observed between climatic and topographic parameters and malaria. Prioritizing the risk zones and identified risk factors will assist in cost effective targeting of malaria interventions and may contribute to a further reduction in malaria burden in the region.

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HEMOGLOBIN C TRAIT PROVIDES PROTECTION FROM CLINICAL FALCIPARUM MALARIA COMPARABLE TO THAT PROVIDED BY HEMOGLOBIN S TRAIT IN MALIAN CHILDREN

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Hemoglobin (Hb) C trait, like Hb S trait, appears to protect against severe malaria in children. Recent work examining whether Hb C trait also protects against uncomplicated malaria has produced conflicting results. We hypothesized that children with Hb C trait would have a longer time to the first clinical malaria episode than children with Hb AA in a cohort study of malaria incidence in Bandiagara, Mali. Three hundred children aged one to six years were enrolled in a longitudinal follow-up study of malaria incidence that included scheduled monthly blood smears and unscheduled follow-up for sick visits. Hb electrophoresis was measured at baseline. Excluding those participants with mutations for glucose-6-phosphate dehydrogenase deficiency, 216 children had Hb AA, 35 children had Hb AC, nine children had Hb AS, three children had Hb SC, and two children had Hb CC. Children with Hb AC had a longer time to first clinical malaria episode than children with Hb AA (P=0.002; 309 mean malaria-free days versus 227 days). Children with Hb AS also had a longer time to first clinical malaria episode than children with Hb AA (P=0.03; 334 mean malaria-free days versus 227 days). Children with Hb AC had fewer episodes of clinical malaria in a single season than did children with Hb AA (0.2 episodes versus 0.7 episodes, P=0.002). However, children with Hb AC or AS experienced the same number of anemia episodes (Hb<8.4 g/dL) as children with Hb AA. Children with Hb AS experienced more asymptomatic malaria episodes (1.44 episodes versus 0.57 episodes, P=0.009) and a lower cumulative parasitemia than children with Hb AC (P=0.02). Thus, while both Hb C and S traits exerted a protective effect

against clinical malaria episodes, they appeared to do so by distinct mechanisms that differentially affected a subject's response to infecting malaria parasites.

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HIGH BURDEN OF MALARIA IN UGANDAN ADULTS AND INCREASED RISK OF SEVERE MALARIA AND DEATH IN ADULT MEN AS COMPARED TO WOMEN

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Malaria prevention has targeted young children and pregnant women because of the disproportionate burden of disease in these populations. Few studies have assessed the frequency and severity of malaria in adult males. To examine the relative impact of gender and age on malaria outcomes, we conducted a chart review of all individuals admitted with a primary diagnosis of malaria to three Ugandan public hospitals between January 2000 and June 2005. The hospitals included Kabale Hospital (hypoendemic malaria transmission), Mulago Hospital (mesoendemic), and Soroti Hospital (holoendemic). 45,176 charts were reviewed. Adult males ≥14 years accounted for 17.2% (Kabale), 8.1% (Mulago) and 8.6% (Soroti) of all malaria admissions, but 35.3% (Kabale), 14.5% (Mulago) and 16.5% (Soroti) of all deaths in persons admitted with malaria. Among persons <14 years, there was no difference in the risk of severe malaria or death in males as compared to females in any hospital. In contrast, among persons ≥14 years of age, males had a significantly higher risk of severe malaria and death than females at two of the three hospitals (risk expressed as odds ratio, 95% confidence interval): Mulago (severe malaria, 1.31, 1.11-1.55, P=0.001; death, 1.54, 1.25-1.90, P<0.0001) and Soroti (severe malaria, 1.56, 1.34-1.82, P<0.0001; death 2.15, 1.72-2.68, P=<0.0001). Among persons admitted to Mulago Hospital with a primary diagnosis of pneumonia, risk of death was also higher in males than females in persons ≥14 years (1.50, 1.23-1.83, P<0.0001) but lower in males than females for persons <14 years (0.86, 0.76-0.98, P=0.02). Among persons ≥14 years of age hospitalized for malaria in Uganda, males have a significantly greater risk of severe disease and death. Given that adult males are largely neglected in malaria control and prevention efforts, further study is needed to understand the reason for this observation.

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GAMETOCYTE DYNAMICS IN AN AREA WITH SEASONAL MALARIA TRANSMISSION

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Renewed interest in malaria elimination has underscored the need for understanding malaria transmission. Gametocytes, the sexual stage of malaria parasites, are responsible for disseminating malaria infection. To better comprehend the epidemiology of gametocytes, including transmission reservoir and "hotspot", in an area with seasonal malaria transmission, we recruited 250 individuals, from ages of 3 months to 50 years, in Bancoumana, Mali. During one year, study subjects were surveyed for parasite carriage every 4 weeks. Children aged 5-15 years had gametocytes more frequently (8%) during their monthly visits compared with individuals in younger or older age groups (2.5-4%). This might be a consequence of a higher proportion of visits with infection in this age group (24% in 5-15 age group compared 7-13% in other age groups) as opposed to a higher probability of having gametocytes when infected. At the beginning of transmission season (July and August), individuals between 5 and 20 years of age were more likely to carry gametocytes than other individuals. However, at the peak of transmission (September through November), individuals from different age groups also presented gametocytes during scheduled visits. There was a strong correlation between proportions of visits with infection in a compound and proportions of visits with gametocytes in the same compound (P<0.01). There was evidence for familial aggregation of gametocyte positivity during follow-up (odds ratio 4 [95% CI 1.04 - 15.3]) but we could not rule out that this might be solely due to infection aggregation. Three compounds that represented 12.4% of the study population had 35.5% of all visits with gametocytes, mostly because of chronic infections in asymptomatic young children. Taken together, the data suggests that children aged between 5 to 15 years carry gametocytes more frequently; whether this is related to longer average duration of infection or to higher incidence of infection in this age group still requires further investigation. Similarly, the identification of factors present in compounds where most gametocyte positivity clusters would guide studies to understand malaria transmission dynamics and possibly the design of clinical trials to test transmission-blocking interventions.

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PLASMODIUM FALCIPARUM MALARIA HAS INCREASED IN BISSAU IN RECENT YEARS, MAINLY AMONG OLDER CHILDREN

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Plasmodium falciparum malaria was holo-endemic in Guinea-Bissau during the early 1990's.We have undertaken back to back clinical trials at the Bandim Health Centre in Bissau since 1994. The health centre serves the population living within the Bandim Demographic Surveillance Site and has been well staffed throughout the study period. The annual number of children aged <15 years, seeking medical attention with at least 20 *P. falciparum* per 200 leukocytes from 1994 to 2011 (except 2009) were as follows: 116, 214, 211, 172, 125, 377, 266, 301, 256, 343, 180, 172, 109, 40, 141, (no data 2009), 316 and 362. Data are lacking Jan-May 1994 (prior to study start), July, August and Nov 1998 (due to civil war), October 2006 (in-between studies) and the whole of 2009 (laboratory staff were not available). The median age of children the same years were; 48, 58, 47, 46, 43, 58, 61, 65, 57, 68, 60, 64, 61, 59, 79, (no data), 107 and 115 months. There was a significant increase of age between 1994 and 2007 as well as between 2007 and 2011 (non parametric test for trend p<0.0001 for both). The number of children aged 5-15 years with malaria were 188/377 (50%) in 1999, 20/40 (50%) in 2003 and 307/362 (85%) in 2011. The annual total rainfall varied with peaks of 1980 mm and 1839 mm in 2003 and 2010, respectively and a trough of 1085 mm in 2007. The overall decrease of malaria after the war in 1999 until 2007 (377 to 40 cases) is in line with findings in neighbouring The Gambia. The decrease was not due to artemether-lumefantrine, as the drug did not replace efficacious high dose chloroquine until 2008. Contrary to the situation in The Gambia, the number of children with malaria has increased ~9 fold since 2007 (40 to 362 cases). The increase consisted of a doubling (20 to 40) of cases amongst children under 5 years of age and a 15 fold (20 to 307) increase in children aged 5-15 years.

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MALARIA IN PREGNANCY IN SOUTHERN PROVINCE, ZAMBIA

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Malaria in pregnancy (MIP) in areas of stable malaria transmission is responsible for maternal anemia and adverse pregnancy outcomes such as low birth weight and preterm birth. In recent years, Zambia has received substantial funding for malaria and has scaled up activities to control MIP. This study assessed institutional capacity to prevent MIP and the utilization of MIP services in Southern Province of Zambia. We conducted comprehensive health center (HC) surveys in Southern Province, Zambia. Pregnant women recruited at the same HCs during routine antenatal care to participate in a neonatal study (ZamCAT) were interviewed at the time of recruitment and 4 days post-delivery about their use of MIP services. Of the 90 primary HCs surveyed, only 25.6% had a functional microscope, 16.7 % had supplies to prepare blood smear and 94.4% had rapid diagnostic tests (RDTs). In terms of antimalarials, 96.6% had oral quinine, 96.6% had artemether-lumefantrine and 87.8% had sulfadoxine-pyrimethamine (SP) for intermittent preventive treatment (IPTp) in stock on the day of the survey. Among 9,816 women interviewed, 55.3% reported sleeping under an insecticide-treated net (ITN) on the night before recruitment and 62.7% reported sleeping under ITN with the baby the night before the post-delivery interview. The average number of antenatal visits made by the women was 3.3; however only 52% received the Zambia Ministry of Health -recommended 3 doses of SP during pregnancy. Women who attended facilities that had SP available were 1.2 times more likely to have completed 3 doses of SP during pregnancy compared to women who attended facilities without stock (95% CI: 1.03, 1.40). Despite appropriate stocking of SP and an adequate number of antenatal visits by pregnant women, many women did not receive the recommended number of doses of IPTp, a situation of missed opportunities. An evaluation of factors responsible for the missed opportunities is needed to improve IPTp coverage.

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USING HEALTH MANAGEMENT INFORMATION SYSTEM DATA ON PARASITOLOGICALLY-CONFIRMED MALARIA CASES TO EVALUATE THE EFFECT OF VECTOR CONTROL COVERAGE

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Routine health management information system (HMIS) data are an under-utilized source for evaluating the effect of malaria control program intensity on the malaria morbidity burden. Since 2009, facilities in Zambia have reported both clinical and parasitologically confirmed (by RDT or microscopy) malaria through the HMIS on a monthly basis. We sought to evaluate the association between vector control coverage and monthly confirmed malaria cases at the district level in Zambia for the period 2009-2011. We first used Bayesian geo-statistical models to create smoothed estimates of insecticide-treated net (ITN) ownership from MIS data and to estimate differences in fever treatment-seeking behavior by district from 2009-2011. We incorporated programmatic data on the distribution of ITNs and indoor residual spraying (IRS) to improve district-level coverage estimates. We included mean monthly rainfall and temperature from remote sensing data to control for climate variability, and additionally controlled for differences in reporting and testing by district and month. We then modeled the association between confirmed cases and vector control coverage with conditional autoregressive models in a Bayesian framework to account for spatial and temporal correlation. After adjusting for reporting, total malaria outpatient cases increased from 3.3 million in 2009 to 4.3 million in 2010, and decreased to 3.8 million in 2011. Confirmed cases represented 29% of total cases in 2009, the first year parasitological confirmations were recorded in the HMIS, 30% in 2010, and 48% in 2011. After controlling for reporting, testing, climate, and district level factors influencing treatment seeking, we estimate that an increase in district level ITN coverage of 1 ITN per household is associated on average with a 19% reduction in population-standardized confirmed case incidence. We did not find an association with IRS. HMIS data, if improved through comprehensive parasitologically confirmed-case reporting, can become an important data source for evaluating associations between malaria program scale-up and spatial and temporal trends in disease burden.

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CLINICAL AND LABORATORY FEATURES OF SEVERE MALARIA IN PERU

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Malaria is a vertor-borne disease considered as one of the main public health concerns by the World Health Organization (WHO). It cause close to 1 million deaths annually. For this reason, WHO established criteria to define severe malaria (SM), in order to reduce the morbidity and mortality. These criteria were based on features of severe malaria cases due *Plasmodium faliparum*, however reports of SM due to *P. vivax* have being rising during the last years. Vivax ans *falciparum* malaria are endemic in Latin America, but the features and prognosis of SM caused by them are poorly characterized. We describe the epidemiological, clinical and laboratory features of patients with SM, in a national reference center of an endemic area of malaria as Peru. Case reports. Patients admitted at Hospital Nacional Cayetano Heredia (HNCH), from 2005 to 2011, with diagnosis of SM according to the 2000 WHO guidelines. The inclusion criteria was to have a full medical record. We identified 40 cases of SM, from which 34 full medical records were available. The mean age for these patients was 39 years (range from 14-64 years) and the male/female ratio was 2.8. Most of the cases came from the Amazon region (47.1%), and few imported cases from Africa (14.7%). *P. vivax* was the most common agent identified in 70.6% of our patients, followed by *P. falciparum* (17.6%) and mixed infection (11.8%). Among the criteria of severity showed by this group of patients, 54.2% (13/34) presented with jaundice and hyperbilirubinemia, followed by 47.1% (16/34) with severe thrombocytopenia, 32.4% (11/34) with hyperpyrexia and 14.7% (5/34) with shock. Only one patient, with renal failure, respiratory insufficiency and multifactorial refractory shock, died of SM caused by *P. falciparum*. No fatal case of *P. vivax* was reported. *P. vivax* is a frequent cause of severe malaria in countries of the Latin America region such as Peru, even if it is not the most common agent reported in the worldwide. The most common complications were liver injury, severe thrombocytopenia, hyperpyrexia and shock.

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COSTING A LARGE-SCALE IMPLEMENTATION OF SEASONAL MALARIA CHEMOPREVENTION IN CHILDREN DELIVERED THROUGH COMMUNITY HEALTH WORKERS IN SENEGAL

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Seasonal Malaria Chemoprevention in children (SMC) is a new strategy for malaria control in areas where transmission is strongly seasonal. In Senegal, a pilot implementation of SMC was conducted by four district health teams from 2008 to 2010 in order to evaluate the feasibility of delivering SMC, its safety and effectiveness, when administered on a large scale using community health workers (CHWs). In 2010, SMC was delivered by 46 health-posts to a rural population of 175,000 children under 10 years of age in 1097 villages, and costing data were collected from each health facility in order to estimate the financial and economic costs of delivery. Delivery was coordinated by the head nurse in each health-post who assigned CHWs to a circuit of villages to visit over a 5-day period in September, October and November, to deliver SMC house to house to all children 3-120 months of age. Tools were developed to collect data on costs and resource use at four levels: the project, the district, the health post, and the CHW. Data was collected from both "top-down", and "bottom-up" (using facility-based costs and extensive interviews on resource use). Data were collected from all 46 health-posts after each round of administration. The study takes a provider perspective with a focus on costs of SMC at the district level. Each health-post employed from 4-68 CHWs and delivery each month took from 2-5 days. High coverage was achieved with about 90% of eligible children treated each month. When the financial cost of delivery was estimated, it cost $233,714 to administer SMC to a population of 175,000 children under 10 years of age at a cost of $0.50 per course. The main cost driver was the incentives paid to CHWs (44%). High coverage of SMC can be achieved at moderate cost. As SMC is now a recommendation from the World Health Organization and each year CHWs may visit households a number of times for distribution of Vitamin A, bednets, mass vaccination and other programs, this will be an opportunities for economies of scope by combining SMC with delivery of other interventions.

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OPERATIONAL METHODS TO OBTAIN GEOLOCATION INFORMATION TO TRACK COMMON DISEASES FOR PATIENTS PRESENTING AT HEALTH FACILITIES IN AREAS WHERE ADDRESSES ARE NOT AVAILABLE: A CROSS-SECTIONAL SURVEY IN FIVE HEALTH FACILITIES IN THE WESTERN KENYAN HIGHLANDS

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The spatial distribution of cases is an important component of understanding the epidemiology of diseases, including malaria, and is valuable in planning and evaluating disease control. Spatial surveillance of cases facilitates targeted control, monitoring for potential epidemics, and evaluation of spatially heterogeneous transmission levels. In countries where no organized network or geocoded database exists, locating where patients come from can be problematic. Obtaining individual coordinates for a health facility attendee is operationally unattractive and less labour intensive methods should be developed that can accurately locate individuals. Such a system would facilitate research and enable disease control interventions to be targeted. To do this, we explored operational approaches to geolocate health facility attendees and determined their relative accuracy. We conducted a cross-sectional survey for malaria in 5 health facilities in the Western Kenyan highlands in October 2011. Of the 1659 people sampled, approximately 30% were followed-up to their compound with coordinates recorded. Information on various geolocation strategies was collected: 1) nearest landmarks to the compound as indicated by the patient, 2) patients identifying the names of heads of compound and 3) of nearest neighbours as well as 4) asking patients to indicate their area of residence on a poster sized satellite image. The effectiveness of the methods was assessed using ArcGIS to create zones around the landmarks where people are more likely to come. A database from previous studies in the area was used as a baseline and the proportion of participants followed-up during the health facility survey that were correctly located was calculated. Preliminary results indicate that of the names of the head of compound and nearest neighbors that were matched, 60% of patients were geolocated to within less than 500 meters of their compound. The results on the optimum approach, or combination of approaches to achieve the most accurate method to the finest possible resolution as assessed by spatial area and population density will be discussed.

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CHANGING OF MALARIA PREVALENCE AND AGE OF INFECTED CHILDREN IN DIFFERENT AREAS OF GABON FROM 2005 TO 2011

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In Libreville, the capital city of Gabon, a reduction of malaria prevalence and a trend towards a higher risk of *Plasmodium falciparum* infection in children aged more than five years have been reported after new malaria control strategies implementation. With the support of Global Fund allocations, Malaria national control program organized the deployment of bednets and Artemisinin based combination therapeutic within the country from 2005. The aim of the study was to estimate the disease burden among children and to characterise malaria transmission intensities based on $PfPr_{2-10}$ in various areas of Gabon. Prospective cross sectional surveys were conducted at the Malaria Clinical Research Unit in Libreville and in four public health facilities at Melen, Port_Gentil, Oyem and Owendo. Febrile pediatric patients, aged less than 11 years old were screened for malaria using microscopic examination. A total of 14293 febrile children were enrolled; 78.5% were less than five years. Between 2005 and 2008, there was a significant drop of malaria prevalence from 35.1% to 16.8%; followed by a raise reaching 26.6% in 2011. Before 2011, $PfPR_{2-10}$ was low in urban areas: 20% at Libreville in 2005 and 2008 and under 5% in 2005 at Port-Gentil. In the rural and semi urban areas of Oyem and Melen, it was above 40.0%. The mean age of infected patients, increased from 37.0 to 48.0 months between 2005 and 2008. From 2008, children above 5 years old were the most infected in all sites. The risk of being infected in this group was 3.21 fold to 5.05 fold higher in urban areas. These data confirm a shift in the age of infected patients towards older children and a large heterogeneity of malaria epidemiology suggesting the need to maintain malaria control strategies in Gabon; and to redefine their implementation throughout the country.

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THE IMPACT OF ACADEMIC DETAILING ON PRESCRIBING AND ACCESSIBILITY OF ACTS IN THE PRIVATE SECTOR IN MADAGASCAR

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Madagascar is participating in the first phase of the Affordable Medicines Facility for malaria, a multi-national subsidy for artemisinin-based combination therapies (ACTs). It is unclear, however, whether subsidized drugs will reach rural areas of the country without additional intervention. We piloted a supporting intervention to encourage prescribing, stocking and purchasing of subsidized ACTs in rural areas of Madagascar by employing "academic detailers" to share scientifically accurate knowledge about ACT effectiveness with doctors and shopkeepers. Baseline cross-sectional surveys on factors related to prescription practices and antimalarial stocking were conducted in five regions of Madagascar in July 2011, covering 160 medical providers and 234 outlets. Additionally, exit interviews were conducted with antimalarial drug shoppers at 128 outlets to identify drug choice. Doctors and outlets in intervention regions were visited by academic detailers with educational ACT materials from October 2011 to March 2012. At baseline, 80.9% of urban outlets were stocked with subsidized ACTs compared to 50.3% of rural outlets. About 80% of providers reported ever prescribing ACTs. Of 279 customers interviewed at outlets that stocked subsidized ACTs, only 27% purchased them. Logistic regression models suggested purchase decisions were predicted by ACT awareness, urban versus rural location, and whether or not the outlet was visited by a representative for subsidized ACTs in the six months prior to the interview. These results suggest the potential for a low-cost intervention involving academic detailers to improve the proportion of treatment-seekers who receive effective antimalarial drugs.

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MALARIA AND ANEMIA PREVALENCE AND INSECTICIDAL NET OWNERSHIP AND USE IN PLATEAU AND ABIA STATES, NIGERIA (2010): RESULTS FROM REPRESENTATIVE HOUSEHOLD SURVEYS

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There have been few recent surveys of malaria prevalence and net coverage in Nigeria. In September 2010, The Carter Center worked with the ministries of health of Abia (Southeast Nigeria) and Plateau (North Central Nigeria) states to conduct a modified Malaria Indicator Survey prior to mass LLIN distribution. In 58 systematically selected clusters (census enumeration areas or segments thereof) of 25 households per state, the average household size was 4.4 persons in Abia (1305 households, 5754 persons) and 6.2 in Plateau (1337 households, 8312 persons). All children <10 years of age were tested for malaria and anemia, and persons of all ages in every third household were tested for malaria. The percentage of households owning ≥ 1 net was much lower in Abia (10.2%) than Plateau (34.8%). The majority of nets were LLIN: 68% (N=123) in Abia and 89.6% (N=489) in Plateau. The percentage of persons using nets the previous night were: Abia: 3.4% of all ages, 6.0% of children under 5 years and 3.6% of pregnant women; Plateau: 14.7% of all ages, 19.1% of children under 5 years, and 21.0% of pregnant women. Crude malaria prevalence by RDT was 36.2% in Abia (95% CI 30.5-41.8, N=2619) and 40.5% in Plateau (95% CI 33.7-47.7, N=4242). Age specific prevalence peaked in the 5- 9 year age group at 47.5% in Abia and 58.9% in Plateau, with second highest prevalence among 10-14 year-olds (Abia 43.0%, Plateau 50.1%). The percentage of children <10 with moderate to severe anemia (hemoglobin < 8 g/dl) was higher in Abia (13.2%, 95% CI 10.3-16.8%, N=1556) than Plateau (5.1%, 95% CI 3.9-6.5%, N=2835). The results reveal high malaria prevalence in these states, and low baseline net ownership. Additional work is needed to explain the fact that anemia prevalence is higher in Abia, though malaria prevalence is comparable to Plateau. A possible contributing factor could be differences in treatment coverage for neglected tropical diseases. Nigeria's universal coverage distribution policy and on going national LLIN distribution campaigns should increase access to LLIN among children 5-14 years of age, but other determinants of use in this age group remain poorly understood.

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LOOKING FOR GOLD, FINDING MALARIA: 2011 MALARIA SURVEILLANCE IN GOLD MINERS' COMMUNITIES IN SURINAME

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Despite the marked reduction of malaria incidence in Suriname, malaria continues to affect the migrants' population (n= 15,000) involved in gold mining. Miners have been trained in the use of RDTs and treatment of

uncomplicated malaria to provide services in their communities. Blood films are prepared for the quality control of all RDTs performed. They report to the Tourtonne laboratory (TL). The TL in the epicenter of the Brazilian gold miners' community in the city is the other component of malaria surveillance in gold miners' communities. The TL staff executes Active Case Detection Campaigns on a regular basis in gold mining areas. The surveillance data serves as the basis of this paper. In 2011, 646 cases were recorded, representing a decrease of 54% (p< 0.0002) from the 1403 recorded in 2010. *Plasmodium falciparum*, *P. vivax* and *P. malariae* were identified in 42.7%, 49.8% and 2.5% of cases respectively. 5.0% had a mixed infection. 484 (75%) cases were imported; the 162 autochthonous cases signify a reduction of 66.3% compared to the 480 reported in 2010. Of the autochthonous cases, 83 (51.2%) were acquired in the Lawa region, 48 (29.7%) around the Lake, Tapanahoni had the lowest number of casses 1 (0.6%). The 162 cases were dispersed over 44 locations. Only 3 (6.8%) locations, all on the Lawa River had more than 10 cases; 47.7% of the locations had only 1 malaria case in 2011. The mean prevalence measured during ACDs was 1.9% (0% - 6.0%). The SPR was 15.1%, ABER 19.9% and API 10.8 per 1000. 97.4% of the infections occurred in Brazilians. 22 cases were reported in pregnant women of which 6 were *P. vivax* relapse. One possible explanation for the tremendous reduction in malaria cases from 2010 to 2011 could be the fact that LLINs have been distributed in 2010 among the populations at risk in the gold mining areas. We have to find innovative ways including cross-border cooperation to deal with the high incidence of imported cases in Suriname.

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THE ESSENCE OF ACCURATE SURVEILLANCE IN A LOW INCIDENCE ERA: REASSESSING AUTOCHTHONOUS CASES THROUGH MALARIA CASE INVESTIGATION IN SURINAME

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The persistent low prevalence measured at any time during 2010 and the low incidence through 2010 of autochthonous cases recorded by the malaria notification points in mining areas brought the authors to the hypothesis that malaria transmission in Suriname is lower than is being captured by the regular surveillance system at the Tourtonne laboratory (TL) in the city. The Malaria Case Investigation form (CI) used by the Bureau of Public Health extended with questions relevant to gold miners was introduced at the TL in February 2011. The CI form captures amongst others, a detailed travel history. The travel history, malaria endemicity and the incubation period for the different species were taken into account to classify the cases either as imported or autochthonous. To test the hypothesis the classification by CI was compared to the classification by general surveillance. 415 malaria cases were diagnosed at TL from February through December 2011. According to the regular surveillance 94 cases were classified as autochthonous. 376 forms were completed, representing 47.2% of all cases (n=797) diagnosed in Suriname. 53 (n=376) cases were classified as autochthonous based on the CI forms. The proportion of autochthonous cases 14.1% based on the CI form was lower (p< 0.003) than the proportion (22.7%) calculated from the general surveillance data. Several possible explanations might account for this difference, including the fact that *Plasmodium vivax* with the ability to relapse if not treated radically, is the predominant infection in Suriname. Self-medication could suppress clinical symptoms and favors the relapse of *P. vivax* and recrudescence of *P. falciparum*. If the patient acquiring an infection abroad stays long enough in Suriname and develops symptoms, the infection might erroneously be recorded as an autochthonous case. Since information on the possible location of transmission is identically captured by all the malaria surveillance systems in Suriname, the authors assume that the over estimation of autochthonous cases is country wide.

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MALARIA CASE INVESTIGATION AT THE TOURTONNE LABORATORY

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Malaria incidence in Suriname has decreased tremendously in the past decade. In order to get a better understanding of the malaria epidemiology and the habits of the persons at risk the need for detailed information on every case becomes more pressing. The Malaria Case Investigation form (CI) used by the Bureau of Public Health extended with questions relevant to gold miners was introduced at the Tourtonne Laboratory in February 2011. The CI form captures amongst others, detailed information on the travel history, symptoms and medical history. Also basic knowledge on malaria prevention is evaluated and the health seeking behavior is assessed. 415 malaria cases were diagnosed at TL from February through December 2011. 376 (90.6%) forms were completed, representing 47.2% of all cases diagnosed in Suriname. Vendors (35%) and gold miners (21.4%) were the groups most affected; CSW 3% were the group least affected. The mean interval between onset of symptoms (Sx) and testing was 5.9 days. The mean interval between onset of Sx and treatment was 6 days. 54.8% (n=312) of the patients used self-treatment. 34.1% (n=314) did not know that malaria is transmitted by a mosquito. 49.4% did not know how to protect oneself against malaria. 13.3% (n=369) used a bed net. Conveying the importance of adhering to appropriate preventive measures for malaria and seeking early detection and effective treatment is a prerequisite to sustain the reduction of malaria. The use of ACT for self-treatment is a concern since it contributes to the emergence and spread of resistance.

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MALARIA IN PREGNANCY IN RWANDA AS THE COUNTRY TARGETS PRE-ELIMINATION

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Rwanda has made strides toward lowering malaria transmission with universal coverage of long-lasting insecticide treated nets and easy access to artemisinin based combination treatment. National prevalence is estimated at 1.4% among children 6-59 months and 0.7% among women aged 15-49 years according to the 2010 DHS. Slide positivity rates from the national health management information system continue to drop and yet malaria persists. Pregnant women thus remain vulnerable even as prevalence drops. While Rwanda no longer practices IPTp it is concerned it is interested in offering the best malaria protection to pregnant women. In order to plan appropriately, there is need for a malaria in pregnancy revalence study. Pregnant women were studied at first antenatal care registration visit in low, moderate and relatively higher transmission areas using rapid diagnostic test and microscopy. Ethical clearance was provided by the ethical review board within the Ministry of Health. ANC staff were trained to obtain data during normal client visits. Among nearly 4000 women studied, prevalence with RDT was 2.4% ranging from 6.6% in the higher border districts in the east to 0% in the areas designated as low transmission based on the HMIS. For microscopy the overall prevalence was 1.6% and also varied from 4.5% to 0.1%. RDT positivity showed reducing trend with increasing parity and with LLIN use the night before the interview. Results show need to continue to protect pregnant women and their unborn children in Rwanda through increased use of LLINs and identification and tracking women of low parity.

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PLASMODIUM FALCIPARUM PARASITE CLEARANCE IN PATIENTS TREATED WITH ARTESUNATE-AMODIAQUINE VS. COMPARATOR GROUPS, SUB-SAHARAN AFRICA

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Monitoring response to artemisinin combination therapy (ACT) worldwide is particularly important now that artemisinin resistance is reported in Southeast Asia. Delayed parasite clearance is considered the best practical surrogate for artemisinin resistance. Artesunate-amodiaquine (ASAQ) is the second most widely used ACT. We analysed 11,570 patients (81% children under 5 years of age) from 41 sites from 20 countries sub-Saharan. The median parasite clearance on ASAQ by site varied from one to two days; a third of the patients cleared their parasitaemia on Day 1; the decrease in mean log parasitaemia between Day 0 and Day 1 was -58% (range: -44% to -83%); between Day 0 and Day 2 it was -96% (-77% to -100%). Using multivariate logistic regression with random effects and controlling for treatment, the risk for a delayed parasite clearance (still parasitaemic on Day 2) was higher in children under five (AOR 1.34, 95%CI 1.10-1.63, p=0.004) as well as in patients with higher parasitaemia at enrolment (AOR 2.56, 95%CI 2.26-2.90, p=0.001). No difference was detected between ASAQ and other ACT (artemether-lumefantrine, dihydroartemisin-piperaquine, AS+SP), but non-ACT (AQ, AQ+SP, chloroquine+SP) carried a higher risk of delayed parasite clearance ($p<0.005$ for all comparisons vs. ASAQ). The analysis provides a platform for future comparisons of antimalarial performance across sub-Saharan Africa.

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THE DEMOGRAPHICS OF WITHIN-COUNTRY POPULATION MOVEMENT NETWORKS IN EAST AFRICA: IMPLICATIONS ON MALARIA TRANSMISSION AND CONTROL

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Human population movement plays an important role in the transmission and importation of malaria. Movement between areas of differing transmission may risk importation of infection from high to low transmission zones. Different demographic and socioeconomic groups are likely to have different movement patterns and infection rates and therefore different risks of importing infections upon travel. It is therefore relevant to quantify and compare movement patterns between varying transmission areas, for different sub-populations. At a national level, household surveys and population census data provide records for individual-level migration. Together with malaria endemicity maps, population distribution maps, mathematical models and network analysis tools, Kenyan, Uganda and Tanzanian migration data was analysed to construct within country population movement networks, useful for malaria importation assessment. The models were further stratified for different demographic and socioeconomic groups to identify and compare movement patterns relevant for malaria importation. Network characteristics, such as cumulative degree distributions and network diameter, were calculated to quantify and compare network structure. Movement networks were different between countries and between demographic and socioeconomic groups. Some demographic groups however, were had similar network characteristics. For example, children under 10 years and adults between 15-24 years had overlapping cumulative degree distributions, illustrating that children were likely to move with their parents. After including malaria in the movement analysis, certain population groups were more likely to contribute to imported infections in certain geographical locations. Census and survey data include migration and demographic data useful for nationwide population movement assessments. Together with national malaria maps and quantitative techniques, malaria importation estimates provide a unique evidence base to inform control policy.

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COMMUNITY ACCEPTANCE OF LARVICIDING FOR MALARIA CONTROL IN RURAL TANZANIA

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Larval source management, including the application of larvicides, is a lesser-used intervention for malaria control yet holds promise as a safe, effective, and environmentally sustainable component of a successful integrated vector management strategy. Recent research has supported the feasibility and effectiveness of larviciding in the urban setting of Dar es Salaam, but its application in rural areas remains understudied. One key element of determining feasibility of larviciding in a rural setting is community acceptance of the method. Community acceptance of larviciding in rural east-central Tanzania was assessed through a range of methods in April-May 2011, including surveys of 962 randomly-selected households from 24 villages, 12 focus group discussions, and in-depth interviews with local leaders and community health workers in each village. The household survey found that the majority of household heads surveyed (82.3%) were not familiar with larviciding as a way to control mosquito larvae in water bodies. Most households (93.8%) indicated that they would grant permission for larvicide to be applied in water bodies where mosquitoes breed near their homes based on a brief standardized description of the process. There was a high level of trust in the safety (74.6%) and efficacy of larviciding, both to control mosquitoes around the home (92.6%) and to reduce the risk of malaria infection (92.9%). Survey questions following up on these attitudes using a Likert scale allow for a more nuanced interpretation of villagers' perceptions. Also, in structured key informant interviews, respondents indicated that community members would be receptive to larviciding in the area, but that community sensitization efforts should be a key component of such an intervention. Household surveys indicated a willingness among community members to make a nominal household contribution (1800 TZS on average, or $1.20 USD) every 3 months. Overall the results of the assessments indicate a receptive environment for future efforts directed at larviciding for malaria control in a rural setting in Tanzania.

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EFFECTIVE PARTNERSHIP DURING HOUSEHOLD CAMPAIGN WITH HANG UP OF INSECTICIDE TREATED NETS MAKE NETS AVAILABLE TO PEOPLE IN GHANA

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Malaria continues to be the cause of significant morbidity and mortality in the country. In 2011, there were Cases and attributed to malaria in Ghana. Insecticide treated nets (ITNS) have been shown avert about 50% of malaria cases. In order to increase ownership and use of ITNs, a household door-to-door campaign to distribute ITNS and hang them in households was instituted. A partnership made up the government, multilateral, bilateral, non-governmental agencies, private sector, political heads, chiefs and elders and the community was formed to ensure the implementation of this campaign. To paper is to describe the partnership at play during the hang up campaign. Partnership started right from the planning stage through the implementation to the post implementation evaluation stages. Looking at the financial and technical capabilities of partners, roles were agreed on and assigned to ensure a coordinated activity. Some partners procured specific quantities of ITNS to cover particular sections the country; others provided funds for procuring other logistics, whilst others provided technical support for quantification, registration, supervision and evaluation. The Chiefs and political leaders contributed through advocacy, conflict resolution and transporting the logistics to the needed sites for distribution. The household campaign, which involved community sensitization, training, registration of households, actual hanging, supervision and monitoring has been undertaken in nine out of ten regions in the country. By end of April 2011, 9,683,160 ITNs had been hanged in the homes of 19,366,320 in the country. Through the effective partnership at play, Ghana is likely to achieve universal coverage of ITNS by July 2012. Resources can be mobilized with the appropriate partnership to achieve health targets and objectives.

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INVESTIGATING MALARIA VECTOR-PARASITE GENOTYPE-GENOTYPE INTERACTIONS AND HOW THEY MIGHT INFLUENCE THE USE OF GENETICALLY-MODIFIED MOSQUITOES IN MALARIA CONTROL

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Typically, malaria transmission models do not consider genotypic structure in the mosquito or the *Plasmodium* population. However, there is evidence that the interaction between malaria parasites and their *Anopheles* vectors is dependent on the specific genotype-genotype (g2g) combination. Here, we initially develop a simple 2 vector - 2 parasite malaria model to explore the impact of g2g interactions on transmission dynamics. This model is then extended to include a greater number of vector and parasite types. In particular, we assume that transmission (from human to vectors) and vector mortality rates are specific for each vector-parasite g2g combination. Motivated by results from experimental infections, we consider whether there is an evolutionary trade-off between transmission and virulence (to the vector) for each vector-parasite combination, and the conditions under which both parasite and vector types can co-exist. The more complex model is used to investigate how introducing a genetically-modified (GM) *Anopheles* population into the system affects the abundance of the other mosquitoes and parasites. Specifically, we consider the case where the GM mosquito is refractory to most, but not all, of the parasite strains. We investigate under which conditions it would be possible for the GM mosquito to replace all other *Anopheles* of the same species, or the conditions under which all malaria parasites could be eliminated. Applications of this work will be helpful to assess the feasibility of using GM mosquitoes to reduce or eliminate malaria in the presence of genotype interactions.

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HOW MANY BEDNETS PER HOUSEHOLD NEED TO BE DISTRIBUTED? EVALUATION OF A UNIVERSAL COVERAGE BED NET DISTRIBUTION CAMPAIGN IN FOUR DISTRICTS IN SOFALA PROVINCE, MOZAMBIQUE

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Malaria remains a priority public health problem in Mozambique. The National Malaria Control Program (NMCP) is conducting universal coverage (UC) distribution campaigns for long lasting insecticide-treated nets (LLINs), among other control measures. However, given the lack of a standard UC distribution model, countries embarking on UC distribute different number of LLINs per household. Setting a fixed number of LLINs per household (HH) is a common strategy, with the risk of these being insufficient or excessive to cover all family members or sleeping spaces. The NMCP piloted a new UC distribution model in 4 districts in Sofala Province (Central Mozambique), using information gathered from the community on the HHs composition (sex, age and relation among each HH member) to determine the number of LLINs to be allocated to each HH based on assumed sleeping patterns. The objective of this model was to maximize the efficiency of the LLIN campaign and cover all sleeping spaces. We conducted an evaluation of these sleeping patterns assumptions, the coverage of sleeping spaces with LLINs (ownership coverage) and the individual use of LLINs by household members (usage coverage). A community-based two-stage cluster random cross sectional survey, including 35 clusters and 32 households per cluster, was performed in May 2010, shortly after the LLIN distribution, and one year later, in June 2011, in the area where the UC campaign had been conducted. Informed consent was obtained from the head of each selected HH and a standardized questionnaire was filled out with information on the LLIN ownership and frequency of use as well as the number of HH members and their sleeping patterns. Analysis of data is ongoing and results will be available in October. This information will be used to validate the assumptions of the distribution model (assumed sleeping patterns within a HH) and to evaluate the effectiveness of the model in covering all sleeping spaces with an LLIN right after the distribution campaign and one year later, as well as to assess other uses of the nets.

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INCREASING ACCESS TO MALARIA PREVENTION IN SOUTH SUDAN BY INTEGRATING NET DISTRIBUTION AND INTERMITTENT PREVENTIVE TREATMENT WITH ANTENATAL CARE AND IMMUNIZATIONS

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Increasing access to malaria prevention by integrating malaria control and prevention services with existing services like antenatal care and routine childhood immunizations is feasible in a post-conflict country like South Sudan where malaria is the leading cause of morbidity and mortality. Pregnant women and children under five are especially at risk. Sleeping under a long-lasting insecticide-treated net (LLIN) may reduce child mortality by as much as 20%. Intermittent preventive treatment

(IPT) reduces the risk of malaria during pregnancy, which may cause complications such as anemia or illness for the mother, and low birth weight or spontaneous abortion for the fetus. In 2010, a household survey reported only 51.6% of pregnant women received one dose of IPT, and only 22.7% received the two doses (IPT2) recommended by the World Health Organization. The second phase of the USAID-funded Sudan Health Transformation Project (SHTP II) targets children and pregnant women by distributing LLINs during antenatal care (ANC) visits as well as during routine immunization for children under five. Between April 2010 to December 2011, SHTP II-supported facilities distributed 79,885 LLINs during routine immunization and ANC visits. There were corresponding increases in ANC1, DPT3 (immunization indicator), and LLIN distribution, culminating in a 97% increase in net distribution. Although an influx of refugees during the 2011 referendum resulted in intermittent stock outs of LLINs, numbers of ANC and DPT3 immunizations continued to rise, demonstrating a consistent increase in access to services and awareness. To prevent malaria during pregnancy, IPT2 services were integrated into ANC visits. IPT2 services increased by 38%, from 4,815 to 6,636 after the first two years of the project. A corresponding increase during this time period was noted in both ANC1 and ANC4 visits (7,638 to 10,301, a 35% increase, and 3,284 to 5,743, a 75% increase, respectively), showing a greater access to primary care as well as an increase in the perceived importance of ANC as well as malaria prevention. The conclusion is that is possible to make significant progress on malaria prevention in a challenging post-conflict, fragile state like South Sudan, by focusing on key interventions that can be integrated with existing services like antenatal care and immunization programming.

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BIOINFORMATICS SYSTEMS FOR UNDERSTANDING MALARIA TRANSMISSION AND CONTROL

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Innovative control strategies that target the entire mosquito life cycle may be required to achieve malaria elimination. Researchers need to analyze huge quantities of ecological data collected from multiple experiments to understand malaria transmission for the development of control strategies. The data preparation process for analysis is very time consuming. We present a bioinformatics system for understanding malaria transmission and control that integrates mosquito densities, infectious status, phenotypic observations, and sample archiving with capabilities to securely store and share data. A relational database schema is designed based on commonly used procedures by mosquito entomologists, which are experiment design followed by sample sorting, observation, constitution, and archiving. Our system handles the data preparation process by providing users with the ability (1) To upload raw data using standardized customizable templates, (2) To download cleaned data for analysis, (3) To generate summarized scientific reports, and (4) To archive and share data locally and globally. Our secure bioinformatics system reduces data preparation time, thus increasing research output. The system provides researchers with field and lab mosquito data rich in information such as densities, species type, and infectious status to address different scientific questions. Researchers upload data using customizable templates that handle data collected using different portable or paper based field collection forms but adhering to standardized terminologies. Users can download cleaned data linking a sample from the field, to the lab, and to a storage location with a data dictionary for analysis. Also, researchers are able to share data and/or to generate quick summaries such as mean catches per mosquito species per infectious status. The system is securely accessible online, but users may opt to run the system locally for data uploading, cleaning, and linking. Our relational schema is extensible to store and link other data such as environmental data and easily can be linked to other databases e.g., demographic surveillance systems (DSS). An extensible bionformatics system for understanding malaria transmission and control is developed to increase research output. Our system allows users to store field- and lab-based mosquito data, download them for analysis, and share them, with an ability to generate quick reports.

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EVIDENCE-BASED BEHAVIOR CHANGE COMMUNICATIONS (BCC) ENHANCE LONG-LASTING INSECTICIDAL NET (LLIN) UPTAKE AND UTILIZATION IN SOUTHEAST NIGERIA

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Long-lasting insecticidal net (LLIN) ownership is often the strongest determinant of net use, but having a net does not guarantee use. Net distribution should be accompanied by evidence-based interventions to address other key determinants. To inform the development of BCC strategies, we asked about social and behavioral determinants of net use during a 2010 survey of 1290 adults in 1192 households located within randomly selected clusters in Imo and Ebonyi States (Southeast Nigeria). Knowledge that mosquitoes transmit malaria was widespread (83%), but 66% reported that malaria was only a risk during the rainy season, and 65% that malaria is caused by eating certain foods. Though 72% reported that LLINs protect against mosquito bites, only 15% mentioned malaria prevention as a benefit. When asked about disadvantages of LLINs, 42% said there are none, but 15% said they were hot and 5% that they cause allergies. More people agreed with the statement that LLINs are safe to sleep under (90%), than that it is safe to hang them where you store food (54%). Only 2.4% knew that LLINs do not need re-treatment. Nets have some negative connotations: 39% agreed that they are "old fashioned;" 33% that they are for poor farmers; and 27% that they are a Western plot to reduce African populations. Low literacy (46%), limited comprehension of languages used for malaria communications, and widespread distrust of many sources of information suggested that home visits by trusted community members would be the most appropriate channel for BCC. The data informed the development of a community-based net monitoring and BCC intervention piloted in six sentinel villages in Ebonyi State in 2011 to improve LLIN ownership, use and care. We stressed the safety and effectiveness of LLINs for both malaria and lymphatic filariasis prevention, and taught skills to make it easier to hang nets at the appropriate height, over any sleeping space. Messages were tailored to fit household behaviors and barriers to use. After six months, 100% of households owned ≥ 1 net (N=1240); 95% of nets were hanging and 94% had been used the previous night (N=2982). 97% of people reported net use (N=5912). All were statistically significant improvements from baseline. Household net data collected by community volunteers provided motivation and direction for an LLIN "mop-up" campaign. This strategy can be modified for implementation by community directed distributors of treatments for neglected tropical diseases.

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THE AFFORDABLE MEDICINES FACILITY-MALARIA (AMFM): ARE REMOTE AREAS BENEFITING FROM THE INTERVENTION?

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In most cases, remote areas are less likely to be covered by health interventions despite often exhibiting the worst health indicators. One aim of the Affordable Medicines Facility - malaria (AMFm) is to ensure that people in remote areas have access to effective and affordable malaria treatment by making subsidized quality - assured artemisinin-based combination therapies (ACTs) available in these areas. AMFm, hosted by the Global Fund to Fight AIDS, Tuberculosis and Malaria, is a financing mechanism which subsidizes quality-assured ACTs for distribution to the public and private sectors, complemented by supporting interventions to promote rational drug use. AMFm has been in operation since mid-2010 in eight national-scale operational pilots in Ghana, Kenya, Madagascar, Niger, Nigeria, Tanzania mainland, Uganda and Zanzibar. By March 2012, over 220 million co-paid ACT treatment doses had been ordered. The Independent Evaluation of AMFm Phase 1 was commissioned by the Global Fund to assess the impact of AMFm on availability, price, market share and use of quality-assured ACTs in all the operational pilots. The assessment is based on a pre- and post-test design with detailed documentation of the implementation process and context, treating each pilot independently. In each pilot, a nationally representative survey of outlets stocking antimalarial medicines was conducted at the baseline (2009/10) and the endline (2011). At the endline, an additional sample of outlets was selected in remote areas in Kenya and Ghana, where availability, price and market share of quality-assured ACTs were measured. Areas were classified by remoteness based on an index computed from estimated travel times to three levels of service centers. The composite index was computed from the sum of the standardized travel times which was used to generate remoteness quintiles with areas in 4th and 5th quintiles considered remote. The number of outlets screened in non-remote and remote areas, respectively, was 501 and 194 in Ghana and 9,980 and 2,353 in Kenya. We compare remote and non-remote areas in each country with respect to availability, price and market share of quality-assured ACTs. The significance of the differences is assessed using Chi-squared tests for proportions and Wilcoxon rank tests for price indicators, expressed as medians.

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INSULIN SIGNALING IN THE MOSQUITO: UNDERSTANDING AKT PHYSIOLOGY IN THE FAT BODY REGULATION AT THE MOLECULAR LEVEL

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Lifespan is a key factor in determining the transmission efficiency of mosquito borne diseases. Finding a novel mechanism affecting mosquito lifespan could be a valuable tool to control mosquito-borne disease transmission. In mosquitoes, the insulin/insulin growth factor 1 signaling (IIS) cascade regulates lifespan, reproduction, and innate immunity. To better understand the impact of IIS in mosquitoes we induced IIS in the fat body of transgenic Anopholes stephensi mosquitoes. To accomplish this we used the vitellogenin promoter to express a myristoylated form of An. stephensi Akt (AsteAkt), a key component of the IIS cascade. Myr-AsteAkt transcript and protein expression occurred as expected with expression only in the fat body, following a bloodmeal. We characterized how changes to IIS specifically in the fat body effects egg production during multiple reproductive cycles and the impact is has on mosquito lifespan. Although myr-AsteAkt expression had little effect on total egg production, lifespan was significantly extended in the transgenic mosquitoes, an effect that was opposite of the anticipated result. Ongoing work on this transgenic mosquito may yield unique insights into how IIS regulates lifespan in mosquitoes and other eukaryotes.

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CHARACTERIZATION OF CARBONIC ANHYDRASES AND ION REGULATORY PROTEINS IN *AEDES AEGYPTI* FEMALE MOSQUITOES PRE- AND POST-BLOOD MEAL

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Mosquitoes represent a major threat to human health due to their capacity to spread diseases, such as malaria and dengue, to both humans and livestock. Blood feeding plays an important role in reproduction and pathogen transmission. Blood meals represent a significant challenge to digestive and ion regulatory processing mechanisms due to the protein, ion, water, and carbon dioxide-rich nature of the blood. This nutrient rich meal needs to be processed during and shortly after a blood meal to facilitate post-blood-meal flight and prevent toxic levels of sodium and CO_2 from remaining in the mosquito. This research determines the respective roles ion transport proteins and carbonic anhydrases play in ion transport and pH maintenance post-blood meal, specifically in the female midgut and hindgut tissues. The ion transporters analyzed include sodium proton antiporters, sodium dependent anion exchangers, and chloride-bicarbonate exchangers. The carbonic anhydrases analyzed fall into the alpha carbonic anhydrase family, with two genes at the focus of our studies. Immunohistochemical analyses reveal that CA9 is localized to the anterior and posterior midgut of the adult, while CA10 is localized to the nervous system and the hearing organ, the Johnston's organ. Immunocytochemical analyses also indicate that the sodium proton antiporter, NHA1, is localized to the apical membranes of the ileum and the stellate cells of the malpighian tubules. We hypothesize that if a female mosquito is fed blood, the expression of ion transport proteins and carbonic anhydrases in the gut will modulate in such a way as to maintain the alkaline pH within the posterior midgut and rapidly transport sodium out of the gut lumen and into the hemolymph. Also, we hypothesize that if ion regulatory or carbonic anhydrase gene expression is perturbed via reverse genetics, the gut and ion regulatory systems will not be able to properly digest the blood meal and regulate ion secretion, thus reducing reproductive capacity and fitness.

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CLIP-SERINE PROTEINASE CLIPB8 SUPPLEMENTS A SRPN2/CLIPB9 REGULATORY UNIT THAT CONTROLS MELANIZATION IN AFRICAN MALARIA MOSQUITO, *ANOPHELES GAMBIAE*

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Melanization immune response encapsulates and kills invading pathogens in insects and other arthropods. Melanization is regulated by the activation of prophenoloxidase (PPO), which is controlled by a proteinase cascade and its serpin inhibitors. To date, the molecular composition of this system is partially understood especially in mosquitoes. Recently, a regulatory unit of melanization in *Anopheles gambiae* was documented comprising an inhibitory serpin-clip-serine proteinase pair: serpin2-CLIPB9. Partial reversion of SRPN2 phenotypes in melanotic tumor formation and adult survival by SRPN2/CLIPB9 double knockdown suggests other target proteinases of SRPN2 in regulating melanization. Here we report that

CLIPB8 is identified as a target proteinase of SRPN2 and supplements SRPN2/CLIPB9 regulatory unit in controlling melanization in *An. gambiae*. Heterologously expressed SRPN2 forms a complex with activated recombinant proCLIPB8 and directly inhibits CLIPB8 activity *in vitro*. Similar as CLIPB9, double knockdown SRPN2 and CLIPB8 also partially reversed the pleiotrophic phenotype induced by SRPN2 silencing both in adult survival and melanotic tumor formation. Differently, CLIPB8 does not cleave and does not activate PPO *in vitro* as CLIPB9 did either by using purified *M. sexta* PPO or *M. sexta* plasma. Biochemical analysis showed that CLIPB8 and CLIPB9 can not activate each other *in vitro*. In addition, reverse genetic analysis by triple knockdown of SRPN2, CLIPB8, and CLIPB9 did not show accumulative effect in reverting the pleiotrophic phenotype by SRPN2 silencing. These results suggest CLIPB8 is on the further upstream of CLIPB9 in activation of melanization.

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A MULTIPLEX REAL-TIME PCR ASSAY FOR DETECTION AND QUANTIFICATION OF *PLASMODIUM* SPP INFECTION IN MALARIA VECTORS

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The enzyme linked immunosorbent assay specific for circumsporozoite protein (CSP-ELISA) is the gold standard method for the detection of malaria parasites in the vector despite several limitations. Here, we developed a new multiplex PCR-based method to detect and quantify the mixed infection rates of *Plasmodium* species in the African malaria vectors to better estimate the level of parasite infection in field populations and to ensure more accurate evaluation of the level of transmission following the implementation of vector control interventions. TaqMan duplex real-time PCR was first evaluated using different ranges of plasmids. The efficiency of real-time PCR was compared with the CSP ELISA using field caught *Anopheles gambiae* and *An. funestus* mosquitoes collected from two localities in southern Benin. Finaly, quantification of DNA of *Plasmodium* spp was performed and normalized using a housekeeping gene RS7. A total of 200 mosquito samples (100 An.gambiae and 100 An.funestus) were used to develop and validate the RT-PCR method. The validation of these oligonucleotides this technique on the mosquito homogenates showed that RT-qPCR was more sensitive than the ELISA-CSP for the detection of *P. falciparum* (RT-PCR RT-PCR= 97% and CSP (RT-PCR RT-PCR= 97% and CSP-Elisa=87%). These results indicated high specificity of the multiplex real-time PCR to detect the other *Plasmodium* species (notably *P. malariae* and *P. ovale*) in anophelinae mosquitoes. The relative quantification shows that the amount of DNA varies between 3 and 90 copy number/ng per samples. The average number of copies / ng in *An. gambiae* is (28.35767) and (7.16700) in An. funestus (p-value = 0.1045). This study describes a new method for the detection and quantification of the four *Plasmodium* species in the African malaria vectors. This will ensure a better diagnostic of malaria parasite's infection in field populations and allow for new basic research on the fitness cost associated with malaria infection during the life of the mosquito.

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PARAQUAT FEEDING FOR STUDY OF MOSQUITO DEFENSE CAPACITY AGAINST OXIDATIVE STRESS

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Anautogenous female mosquitoes take blood meals for egg production. Digestion of hemoglobins is accompanied with heme associated oxidative stress, which is potentially detrimental to lipids, proteins and DNA. As an adaptation, mosquitoes have evolved certain antioxidant mechanisms to cope with this oxidative stress. However, little is known about the extent of defense capacity that mosquitoes have. Paraquat is an herbicide known to causes extensive damage to the mitochondria through the production of free radicals and oxidative stress. In this study we used Paraquat feeding to add extra oxidative stress to the mosquitoes, and examine the anti-oxidant capacity in the gut ecosystem. Mosquitoes were fed on sugar diet with different concentration of Paraquat (2mM, 10mM and 20mM) after emergence. Paraquat causes mosquito death in a dose dependent manner. Interestingly, blood feeding increased the mortality of mosquitoes that had been fed on 2 mM Paraquat, suggesting that the bloodmeal increased the stress to a level that exceeds the defense capacity of mosquitoes. The expression patterns of mosquito and bacterial catalase and SOD, and bacterial AhpC, Paraquat inducible protein A and B genes were assayed by qPCR. These mosquito and microbial anti-oxidant genes responded to the stress in various settings, such as blood-fed, Paraquat-fed and Paraquat-plus blood-fed mosquitoes. The data suggest that gut redox homeostasis is managed collaboratively by both mosquito and its microbial community.

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TRANSCRIPTIONAL MEDIATORS KTO AND SKD ARE INVOLVED IN THE REGULATION OF THE IMD PATHWAY ANTI-*PLASMODIUM* DEFENSES IN *ANOPHELES GAMBIAE*

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Malaria is responsible for the deaths of over one million people annually. *Anopheles* mosquitoes are the main vectors for the malarial parasites. We have shown that the IMD pathway is the most important arm used by the mosquitoes to resist infection with the human malaria parasite *Plasmodium falciparum*. In this study, we showed that the transcriptional mediators Kto and Skd are involved in the regulation of the IMD pathway. Transcriptional mediators serve as transcriptional co-activators, which are a group of evolutionally conserved proteins that can form complexes to bridge regulatory regions to the RNA polymerase II initiation complex in eukaryotic cells. Studies with *Drosophila*, zebrafish and *Caenorhabditis elegans* have shown that Kto and Skd are required for several specific developmental processes. Here we show that knocking down Kto and Skd in the *Anopheles gambiae* cell line down-regulate the expression level of Cec1 which is controlled by the IMD pathway. However, Kto and Skd are not transcriptional co-activators of Rel2 and are not involved in the transcription of the main IMD pathway components. Silencing the two genes *in vivo* would lead to increased susceptibility of mosquitoes to bacterial and *P. falciparum* infection, but not to infection with P. berghei. Together the results suggest that Kto and Skd are involved in the regulation of the IMD pathway, which is crucial for the mosquito's defense against *P. falciparum*.

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FRAGMENTATION MECHANISMS OF ARGININE ISOBUTYL ESTER APPLIED TO ARGININE QUANTIFICATION IN EXCRETA FROM INDIVIDUAL *AEDES AEGYPTI* FEMALES

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Our laboratory is interested in uncovering the metabolic regulation of argininolysis and uricolysis in mosquitoes. For this purpose, it is necessary to have a rapid and efficient method to monitor arginine (Arg) levels in excreta from individual *A. aegypti* females. Thus, the fragmentation patterns of the isobutyl esters of Arg and $^{15}N_2$-Arg (labeled at the guanidino group) were studied by electrospray ionization-tandem mass spectrometry, and fragmentation pathways not described before were characterized. In addition, Arg, $^{18}O_2$-Arg, $^{15}N_2$-Arg and $^{15}N_2$-$^{18}O_2$-Arg were analyzed to elucidate some of the minor fragments in greater detail. Mosquito excreta from individual females were collected before and at different times after feeding a blood meal, mixed with $^{15}N_2$-Arg, an internal standard, and derivatized as isobutyl esters. Based on the fragmentation mechanism of Arg standards studied by MS^2 and MS^3, Arg levels in the mosquito excreta were analyzed by multiple-reaction monitoring (MRM) in a triple-quadrupole mass spectrometer. Arg excretion was quantified at 1, 6, 12, 18, 24, 36, 48, 72, 96 and 120 h before and after feeding female mosquitoes with a bovine blood meal. As expected, Arg is not present in the sugar-fed female excreta and only a very small amount is observed in blood-fed female excreta at the beginning of the time course. At 12 h, the Arg concentration is approximately 20 nmol/female mosquito. This value increases significantly during the time course, reaching the highest levels between 36 and 48 h (about 60 nmol/female) and remains constant through the end of the time course (120 h after a blood meal). These data correlate well with the periods of intense blood meal digestion and maximal excretion of nitrogen compounds in the blood-fed females. The quantification of Arg by mass spectrometry provides a rapid, sensitive and accurate method to investigate the metabolic regulation of nitrogen wastes in individual *A. aegypti* females.

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MOLECULAR AND FUNCTIONAL CHARACTERIZATION OF INWARD-RECTIFYING POTASSIUM (KIR) CHANNELS IN THE 'KIDNEYS' OF MOSQUITOES: TOWARDS THE DEVELOPMENT OF NEW INSECTICIDES

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The evolution of insecticide resistance in mosquitoes has led to an urgent need to develop new chemical control agents with novel mechanisms of action. In the present study, we evaluate the inward-rectifying potassium (Kir) channels of mosquitoes (Aedes aegpyti) as potential insecticidal targets by characterizing their molecular and functional expression. We focus our study on the Kir channels expressed in the Malpighian tubules, because this renal epithelium is a key component of the mosquito excretory system and has not been exploited as a physiological target for controlling mosquitoes. We show that (1) Malpighian tubules express a combination of at least 3 different Kir channel genes that is distinct among mosquito tissues, (2) at least two of the Kir channels encode barium-sensitive potassium channels when expressed in Xenopus oocytes, and (3) injecting a small-molecule antagonist of Kir channels into mosquitoes elicits desirable sub-lethal effects that are consistent with perturbed Malpighian tubule function.

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THE ROLE OF APOPTOSIS IN DENGUE-2 INFECTION OF THE MOSQUITO VECTOR *AEDES AEGYPTI*

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Aedes aegypti is the primary vector for dengue virus (DENV). An understanding of host-pathogen interaction is important in understanding what factors contribute to vector competence. Our previous global transcriptional analysis has suggested the induction of apoptotic proteins in the involvement of resistance and susceptibility to DENV infection. However the mechanism through which is happens is largely unknown. Here we analyze the possibility that programmed cell death is actively involved in the defense of *A. aegypti* host cells to DENV infection. The effector caspase, *CASPSL2*, has been previously shown to be part of the core apoptotic pathway involved in the response to drug and UV-induced DNA damage in *A. aegypti*. Here we use siRNA interference to show that *CASPSL2* is also involved in apoptotic signaling for DENV-2, and that silencing of this gene affects virus titer at early and late points of infection. Silencing of *CASPL2* also affected dissemination and transmission of the virus. In addition, we investigate the possibility that by delaying programmed cell death in susceptible individuals, DENV-2 can manipulate this process for its benefit.

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P38 MAPK SIGNALING IN *ANOPHELES STEPHENSI*: A MECHANISM FOR TOLERANCE OR RESISTANCE DURING PARASITE INFECTION?

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Among the mitogen-activated protein kinases, p38 MAPK-dependent signaling is critical to the regulation of the balance between resistance and tolerance to infection. However, little is known about the functional biology of p38 MAPK signaling in vector mosquitoes. Our data demonstrated that inhibition of *Anopheles stephensi* p38 MAPK signaling can reduce malaria parasite development, including oocyst burden in the midgut as well as infection prevalence. Further, p38 MAPK signaling regulates a wide variety of known mosquito anti-parasite effector genes in patterns that suggest a balance between tolerance and resistance. This work indicates that the essential roles of p38 MAPK signaling identified in mammals are conserved in mosquitoes. More importantly, however, our data provide new insights into regulatory mechanisms that can be manipulated to control suites of anti-parasite genes as the basis for a novel strategy for the development of transgenic, parasite-resistant mosquitoes.

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CHARACTERIZATION OF A G PROTEIN COUPLED RECEPTOR (GPCR) THAT BINDS TO THE ANTI-*PLASMODIUM* IMMUNE FACTOR FBN9

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In *Anopheles gambiae* mosquitoes, the fibrinogen related protein family (FREP, also known as FBN) is the largest group of pattern recognition receptors. We have previously reported that one of its members FBN9, interacts directly with various species of bacteria and also exhibits anti-*Plasmodium* activity. To further understand the role of FBN9 in the mosquito's innate immune system, a yeast two hybrid screen was performed to identify novel binding partners. In addition to a number of other interacting proteins, we have discovered a G protein coupled receptor (GPCR) that binds to FBN9. Interestingly, this GPCR has been

identified as a rhodopsin receptor (GPROP10) and has no previously described immune related function. Rnai studies show that GPROP10 may also participate in controlling *Plasmodium* development in the mosquito midgut. Here we describe the characterization of GPROP10 and its potential function in the innate immune system of *An. gambiae* mosquito's defense against pathogens.

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TRANSCRIPTOMIC COMPARISON OF LABORATORY AND GEOGRAPHICALLY DISTINCT FIELD-DERIVED *AEDES AEGYPTI* POPULATIONS TO IDENTIFY GENES THAT REGULATE VECTOR COMPETENCE FOR DENGUE VIRUS2

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Dengue virus (DENV) is the most important mosquito-borne virus affecting humans today, and is vectored primarily by the mosquito *Aedes aegypti*. Since no vaccine against DENV is currently available, there is interest in transmission control strategies that target the mosquito vector. Most studies of mosquito immune responses have been performed with the laboratory strains of *Ae. aegypti*, which have been maintained under insectary conditions for decades. As compared to natural mosquito populations, laboratory mosquito strains are exposed to lower doses and a much narrower range of microbes; this together with the genetic bottleneck of a small initial parental population size often results in a loss of genetic variability. Although most field studies have focused on genetic polymorphisms, natural and laboratory mosquito populations are also likely to differ in their transcriptomic responses to pathogen infection, either in terms of the magnitude of gene regulation or in the subsets of regulated genes. We established field colonies of *Ae. aegypti* from geographically-distinct dengue-endemic regions, spanning South America, the Caribbean, and Southeast Asia, and evaluated their and vector competences for DENV2. This analysis identified both refractory and susceptible strains to DENV2 infection. A genome-wide gene expression microarray was then performed to compare the transcriptomes of field-derived strains to our laboratory Rockefeller strain. Several candidate genes were identified that may regulate vector competence in field-derived strains; we are currently functionally testing the role of these genes through RNAi-mediated gene knockdowns. This study will not only provide valuable information about immune gene regulation and usage in natural mosquito populations, but will also allow us to identify novel pathogen recognition receptors and effector genes that control DENV in field mosquitoes.

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HIGH PREVALENCE OF HTLV-1 AND HTLV-2 INFECTIONS IN PERUVIAN AMAZONIAN COMMUNITIES

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HTLV-1 and HTLV-2 infections are distributed worldwide and endemic to regions of Japan, sub-Saharan Africa, the Americas, Melanesia, and the Middle East. Peru has reported HTLV-1 and HTLV-2 infections in indigenous Amazonian populations and among African-Peruvian and mestizo populations. To assess the prevalence, risk factors and neurological manifestations associated with HTLV-1 and -2 infections, we conducted a cross-sectional study of 878 adult participants, ages 15-64 years, from 14 indigenous communities near Pucallpa. 94 (10.7%) participants were infected with HTLV: 56 (59.6%) had HTLV-1, 35 (37.2%) HTLV-2, and 3 (3.2%) were infected with both HTLV-1 and HTLV-2. Seven patients had indeterminate test results and were excluded from further analysis. The median age for all participants was 34 (SD ± 13.8) years. HTLV positive participants were older than HTLV negative participants (mean 43.1 vs. 32.9 years ($p<0.0001$). HTLV-1 and -2 infections increased with age ($p<0.0001$) but decreased for participants aged 50 years or older. Factors significantly associated with HTLV infection included age ≥ 38 years ($p<0.0001$, OR: 3.07), female gender ($p=0.008$, OR: 1.82), illiteracy ($p=0.002$, OR: 2.85), education of 7 years or less ($p<0.0001$, OR 2.22), having had a relative with gait difficulties affecting both legs ($p=0.036$, OR 2.4), prior episode of chronic scabies (significant only for males; $p=0.046$, OR: 2.1), and being pregnant more than four times ($p=0.027$, OR 1.88). Surprisingly, no participant had clinical evidence of HTLV-associated myelopathy/tropical spastic paraparesis (HAM/TSP). To our knowledge, this is one of the highest reported prevalences of HTLV infection among native Amazonian ethnic groups. Although not measured in this study, the high prevalence of helminthic coinfection reported among Amazonian inhabitants may potentially attenuate immune responses and thus impede the development of HAM/TSP.

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AUSTRALIAN ARBOVIRUSES AND A NOVEL RHABDOVIRUS IN ANOPHELINE MOSQUITOES IDENTIFIED USING METAGENOMICS

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Identifying viruses as etiologies of human and animal disease is an important initial step in preventing and treating illness. The success of many previous virus identification strategies has been impeded by the requirement for prior knowledge of the viral genome, which dictates the testing assay. Deep sequencing, by contrast, is a cutting-edge metagenomics technique that detects and characterizes known and unknown viruses in a specimen nonspecifically and with high sensitivity, without prior knowledge of the viral genome. We used deep sequencing to identify virus genomes in pools of mosquitoes from New South Wales, Australia, that were antigenically negative for known Australian flaviviruses and alphaviruses. Full genome characterization and phylogenetic analyses revealed sequences of several viruses in a least one pool each: 1) strains of Liao Ning virus (LNV, *Reovirus*, *Seadornavirus*), heretofore only detected in Indonesia and China where it is the etiological agent of encephalitis in humans, 2) strains of Stretch Lagoon virus (SLOV, *Reovirus*, *Orbivirus*) a mosquito-borne virus that infects livestock and has previously been isolated only in Northern Australia and once in Sydney, and 3) a novel rhabdovirus in *Anopheles annulipes* that diverges by ≈40% at the amino acid level compared to other members of the *Vesiculovirus* genus and probably represents a new species. To our knowledge, this is the first report of LNV outside China, and we extend the distribution of SLOV to central New South Wales. This study highlights the power of metagenomics for identifying novel RNA viruses in field-collected mosquitoes. The new rhabdovirus may eventually be linked to human or veterinary disease, and follow-up epidemiological arbovirus studies will address this possibility.

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LASSA FEVER OUTBREAK INVOLVING HEALTHCARE WORKERS IN TARABA STATE, NIGERIA: MARCH 2012

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Lassa fever is an acute, highly infectious viral haemorrhagic illness caused by Lassa fever virus - a single stranded, RNA virus belonging to the virus family Arenaviridae. The reservoir is Mastomys natalensis. The disease is endemic in West African sub region causing 300,000-500,000 infections annually, with about 500 deaths. In March, 2012, we investigated a reported outbreak of Lassa fever in Taraba State, Nigeria to confirm the outbreak, determine its extent, characterize the outbreak, institute public health actions and make appropriate recommendations. We reviewed hospital records and used IDSR standard case definition for Lassa fever to identify and line-list cases. A suspected case was defined as "any person with severe febrile illness not responsive to the usual causes of fever in the area with or without sore-throat and at least one of the following: bloody stools, vomiting blood, bleeding into the skin, unexplained bleeding from the nose, vagina or eyes". A standardized line-listing form was developed to capture socio-demographic and clinical information of the cases. Various exposure factors including age, gender, occupation and contact history were examined. A total of 35 cases were recorded. Nine of 35 cases were laboratory confirmed (25.7%). Altogether, 14 deaths were recorded giving a case fatality rate of 40%. Majority of the cases belonged to the age group 25-34 years (40%) with females constituting 51% . Most of the cases were healthcare workers (22.9%). The commonest presenting features were fever (85.7%), cough (28.6%), bleeding from orifices or into skin (25.7%) and headache (20%). In addition, the State's Epidemic Management Committee was non-functional resulting in uncoordinated response to the outbreak. There were many exposure factors to Lassa fever such as over-crowding, drying of food items along high ways and bush burning and there was low index of suspicion of Lassa fever among health care workers. Community sensitization and sensitization of health workers in Taraba State on Lassa fever were carried out. There was a confirmed outbreak of Lassa fever in Taraba State mostly affecting healthcare workers. It was recommended that the State should reactivate its Emergency Management Committee, surveillance of Lassa fever should be strengthened, Public/Health workers sensitization activities should be scaled up and records keeping should be improved.

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DIARRHEA INCIDENCE BEFORE AND AFTER ROTAVIRUS VACCINE INTRODUCTION IN NICARAGUA: A PROSPECTIVE, POPULATION-BASED STUDY

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Nicaragua was the first GAVI-eligible country to introduce the pentavalent rotavirus vaccine in 2006. Prior evaluations of the vaccine's effectiveness in developing countries have been performed in health facilities; however, the majority of rotavirus cases are treated in the community. The goal of this study was to examine changes in childhood diarrhea incidence in the community before and after vaccine introduction. We conducted active surveillance for diarrhea episodes using the Health and Demographic Surveillance Site, León to provide simple random population-based samples. Two open cohorts of children were followed, one in the pre-vaccine period, 2001-2003, and the other in the post-vaccine period, 2010-2011. Home interviewers visited households to record each child's characteristics and returned every 2 weeks to record numbers of diarrhea episodes. Poisson regression models were used to compare the incidence rate of diarrhea in the pre- and post-vaccine periods, stratified by age. Because laboratory data were not available for comparison, a "rotavirus-specific" diarrhea surrogate definition was used, based on the literature: greater than 4 stools per 24 hr period with either vomiting or fever or both. We anticipated a decline in rotavirus-specific diarrhea incidence in the post-vaccine period. A total of 726 children were enrolled in the pre-vaccine cohort and were followed for 249 person-years (py); 826 children were enrolled in the post-vaccine cohort and were followed for 563 py. Overall unadjusted diarrhea incidence was lower in the post-vaccine period than in the pre-vaccine period. Rotavirus-specific diarrhea incidence showed a greater decline from the pre-vaccine to the post-vaccine periods: among infants from 0.38 to 0.14 cases per py (p=0.026), among 12-23 month old children from 0.35 to 0.07 cases per py (p=0.001), and among 24-59 month old children from 0.10 to 0.02 cases per py (p=0.002). In conclusion, substantial declines in the incidence of rotavirus-specific diarrhea were observed in the post-vaccine period in this community setting.

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MOLECULAR DETERMINANTS OF MOUSE NEUROVIRULENCE AND MOSQUITO INFECTION FOR WESTERN EQUINE ENCEPHALITIS VIRUS

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Western equine encephalitis virus (WEEV) is a naturally occurring recombinant virus derived from ancestral Sindbis and eastern equine encephalitis viruses. We previously showed that infection of CD-1 mice with WEEV McMillan (McM) and IMP-181 (IMP) isolates resulted in high (~90-100%) and low (0%) mortality, respectively, when virus was delivered by either subcutaneous or aerosol routes. However, relatively little is known about specific virulence determinants of WEEV. We additionally observed that IMP infected Culex tarsalis mosquitoes at a high rate (app. 80%) following ingestion of an infected bloodmeal but these mosquitoes were infected by McM at a much lower rate (10%). To understand the viral determinants generating these phenotypic differences, we characterized the pathogenic phenotypes of McM/IMP chimeras. Exchanging the arginine present at IMP E2 glycoprotein position 214 for the glutamine present at the same position in McM ablated mouse mortality. However, the reciprocal exchange did not confer mouse virulence to the IMP virus. Mosquito infectivity was determined by multiple loci one of which was the same E2-214 amino acid identified above as the mouse virulence determinant . Replacing either IMP E2 amino acid 181 or 214 with the corresponding McM amino acid lowered mosquito infection rates to McM-like levels. As observed during our study of mouse neurovirulence, neither reciprocal exchange conferred mosquito infectivity. The identification of WEEV E2 amino acid 214 as necessary for both IMP mosquito infectivity and McM mouse neurovirulence indicated that they are mutually exclusive phenotypes and suggests an explanation for the lack of human or equine WEEV cases even in the presence of active transmission.

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PROBING THE ROLE OF CD4+ AND CD8+ T-CELLS IN CONTROLLING EARLY INFECTION WITH THE CHIKUNGUNYA CHIKV/IRES CANDIDATE VACCINE AND PROTECTING AGAINST CHIKV CHALLENGE

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Recently, Chikungunya virus (CHIKV), a mosquito-borne alphavirus, re-emerged in Africa and spread to islands in the Indian Ocean, Indian subcontinent, SE Asia and Italy. Viremic travelers have also imported CHIK to the Western hemisphere, which highlights the risk of CHIKV in naïve populations. In addition to the great burden of arthralgic disease, which can persist for months or years, epidemiologic studies estimated case-fatality rates of ~0.1%, principally from neurologic disease in older patients. There are no licensed vaccines or effective therapies. Using the La Reunion strain as the genetic backbone we inserted a picornavirus internal ribosome entry site (IRES) that functions poorly in insect cells, and inactivated the subgenomic promoter which drives overexpression of the structural proteins, to develop a live-attenuated CHIKV vaccine (CHIKV/IRES). This vaccine is highly attenuated yet immunogenic in mouse models and non-human primates, and is incapable of replicating in mosquito cells. In an effort to understand better the contribution of host response to CHIKV/IRES replication at the initial stages of virus infection we are currently conducting a series of studies to determine whether $CD4^+$ and/or $CD8^+$ T cells control virus replication in the A129 mice (deficient in the IFN α/β response). Prior to CHIKV/IRES infection, T cell subsets are depleted and the replication of the virus in the serum and various tissues (brain, spleen, muscles, liver) are monitored over a period of 12 days.In addition, isolated T cell subsets collected on days 0, 3, 6, 9 and 12 are characterized by intracellular cytokine staining using flow cytometry. Furthermore, we are conducting a series of adoptive transfer studies to determine which T cell subset is contributing to the protection afforded by the vaccine against wt CHIKV challenge. Understanding the role of T cell immunity in controlling infection and its contribution to protection will assist our efforts in designing an effective vaccination strategy against CHIKV.

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VACCINE SAFETY AND THE DEVELOPMENT OF A RODENT MODEL OF PERSISTENT CHIKUNGUNYA VIRUS INFECTION

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Chikungunya virus (CHIKV) is a positive sense single stranded RNA virus in the genus Alphavirus and the etiologic agent of several epidemics in Africa, and recently, the Indian subcontinent and Southeast Asia. CHIKV causes a syndrome characterized by rash, fever, and debilitating arthritis. In the more recent outbreaks, CHIKV has begun to manifest more neurologic signs of illness in the elderly and those with co-morbidities. The syndrome is often self-limited; however, many patients develop persistent arthralgia that can last months or years. These characteristics make CHIKV not only important from a human health standpoint, but also from an economic standpoint. Currently, there is no licensed vaccine. Many studies have begun to elucidate the pathogenesis of CHIKV, the mechanism of arthralgia persistence and the role of the adaptive immune response that is poorly understood. In this study, Rag1 KO (Recombination activation gene 1 knockout) mice were inoculated subcutaneously or in the foot pad with 3 log10 PFU of the La Réunion strain of CHIKV (CHIKV-LR) or varying doses of our vaccine candidate CHIKV/IRES (1, 3, or 5 log10 PFU) or the U.S. Army vaccine strain 181/clone25 (3 or 5 log10 PFU). Mice were bled on days 1-8, 14, 28, 42, 56 and 70 after infection and weighed on days 1-14. Tissues were harvested on days 2, 4, 7, 14, 28, 42, 56 and 70. None of the subcutaneously inoculated mice demonstrated clinical signs of illness (e.g., weight loss, lethargy, or scruffy fur). Mice inoculated with CHIKV-LR developed persistent infection. Viremia reached a peak of 4 log10 PFU on day 6 after infection, gradually decreasing to 2 log10 on day 28; no viremia was detected after day 28 post infection. CHIKV-LR also persisted in several organs up to day 42 after infection, but no virus was detected by plaque assay in the organs after day 42 post infection. The brain had inflammation 28 days post infection. These findings are in contrast to both vaccine strains, which never produced detectable viremia or viral persistence in the organs. This study has two key findings: 1) the adaptive immune system is critical for clearance of CHIKV, and 2) the newly developed CHIKV/IRES vaccine strain does not persist even in the absence of T/B cells. The latter point is very important when considering vaccine safety, because many people in developing countries that are exposed to CHIKV are also immunosuppressed due to various conditions (e.g., HIV, malnourishment).

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DEVELOPMENT, VALIDATION, AND FIELD PERFORMANCE OF A FIVE-PLEX REAL-TIME QPCR ASSAY TO DETECT DIARRHEAGENIC RNA VIRUSES

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Several viruses and bacteria are known to cause diarrheal disease in the developing world. Traditional methods such as microscopy or culture are either inappropriate or impossible when dealing with these pathogens. In addition, ELISA-based tests may not possess the sensitivity required to detect these pathogens from stool samples. PCR-based methods are generally more sensitive compared to both traditional and ELISA-based methods. In this work, we developed and validated a 5-plex TaqMan-based real-time qPCR assay targeting diarrheagenic RNA viruses including: Astrovirus, Norovirus GII, Rotavirus, and Sapovirus (types 1,2,4, and 5), and includes an extraction/amplification control based on a non-coding region of MS2 phage. In addition, we also developed a 4-plex TaqMan-based real-time qPCR panel targeting Campylobacter, Salmonella, and Vibrio bacterial species with the Glycoprotein B gene from Phocene Herpes Virus as the extraction/amplification control. The validation process revealed at least five logs of linear range for each viral target, as well as low CV values (range 0.500-1.855%) for within-run precision and moderate CV values (range 2.8%-28.9%) for between-run precision depending on the target. In addition, the assay appears to perform well in stool matrices having varying degrees of inhibition. After validation, the assays were evaluated at five international field sites each using one of three different real-time PCR platforms including the BioRad CFX96, Corbett/Qiagen RotorGene, and the Applied Biosystems ViiA 7. The field evaluations were based upon measures of linearity, limit of detection, within- and between-run precision ("repeatability" and "reproducibility", respectively), as well as accuracy and carry-over studies. Similar to the results from the validation, the field performance evaluation revealed low CV values (range 0.08-7.52%) for within-run precision and moderate CV values (range 2.36-11.06%) for between-run precision analyses. While there were site-to-site and target-to-target differences, overall, the assays performed similarly over the five field sites and similarly between the three real-time PCR platforms.

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SEVERE HEMORRHAGIC FEVER IN STRAIN 13/N GUINEA PIGS INFECTED WITH LUJO VIRUS

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Lujo virus (LUJV) is a novel member of the *Arenaviridae* family that was first identified in 2008 after an outbreak of severe hemorrhagic fever (HF). In what was a small but rapidly progressing outbreak, this previously unknown virus was transmitted from the critically ill index patient to 4 attending healthcare workers. Four persons died during the outbreak, for a total case fatality of 80% (4/5). The suspected rodent source of the initial exposure to LUJV remains a mystery. Because of the ease of transmission, high case fatality, and novel nature of LUJV, we sought to establish an animal model of LUJV HF. Initial attempts in mice failed, but infection of inbred strain 13/N guinea pigs resulted in lethal disease. A total of 41 adult strain 13/N guinea pigs were infected with either wild-type LUJV or a full-length recombinant LUJV. Results demonstrated that strain 13/N guinea pigs provide an excellent model of severe and lethal LUJV HF that closely resembles what is known of the human disease. All infected animals experienced consistent weight loss (3-5% per day) and clinical illness characterized by ocular discharge, ruffled fur, hunched posture, and lethargy. Uniform lethality occurred by 11-16 days post-infection. All animals developed disseminated LUJV infection in various organs (liver, spleen, lung, and kidney), and leukopenia, lymphopenia, thrombocytopenia, coagulopathy, and elevated transaminase levels. Serial euthanasia studies revealed a temporal pattern of virus dissemination and increasing severity of disease, primarily targeting the liver, spleen, lungs, and lower gastrointestinal tract. Establishing an animal LUJV model is an important first step towards understanding the high pathogenicity of LUJV and developing vaccines and antiviral therapeutic drugs for this highly transmissible and lethal emerging pathogen.

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ORAL ROTAVIRUS IMMUNIZATION PROTECTS UNDERNOURISHED WEANLING MICE AGAINST INFECTION DESPITE REDUCED VACCINE SHEDDING AND MODULATED ANTIBODY RESPONSES

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Oral rotavirus vaccines protect against the most common cause of severe childhood diarrhea worldwide, but are less effective in low-income countries. A higher prevalence of undernutrition, reducing both innate and adaptive immunity, may partially explain this disparity. Therefore, we designed mouse experiments to test the hypothesis that undernutrition impairs immune responses to rotavirus vaccine and infection. Wild type BALB/c dams with 10-day-old sucklings were randomized to a standard diet or an isocaloric, multideficient "regional basic diet" (RBD) we have previously shown produces moderate malnutrition and phenocopies key features of human environmental enteropathy, including villous blunting and reduced gut integrity (Ueno et al. *AJP* 2011). We immunized RBD mice and controls at 6 weeks of age with a live oral rotavirus vaccine (RRV) or a vehicle control. We then challenged immunized mice and unimmunized controls with murine rotavirus (EDIM 10^4 SD_{50}) 4 weeks later. Stool and blood were collected after RRV or EDIM challenges to determine viral shedding and antibody responses. RRV shedding in stool following immunization was decreased by 50% in RBD mice vs. controls (15.1 ng/mL vs. 30.8 ng/ml, $P<0.03$), however protection against EDIM was undiminished. Following immunization, RBD mice had 2-fold higher anti-rotavirus serum IgA levels vs. controls. Following infection, unimmunized RBD mice produced 50% lower levels of anti-rotavirus IgG vs. well-nourished controls ($P=0.16$). This was not significant after correcting for marked decreases in total IgG levels in RBD mice). In conclusion, weanling undernutrition alters host immune responses to rotavirus vaccination and infection, but does not mitigate vaccine efficacy. Further research defining the role of malnutrition and other host factors is needed to improve vaccination outcomes in children who bear the greatest risk of disease.

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IRES-BEARING VENEZUELAN EQUINE ENCEPHALITIS VIRUSES ARE POTENTIAL VACCINE CANDIDATES

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Venezuelan equine encephalitis virus (VEEV) is an arbovirus associated with morbidity and mortality in equines and humans across Central and South America. Despite its success in curbing the severity and range of epidemics in the past, the current VEE vaccine, TC-83 is a suboptimal vaccine for the following reasons: (i) unstable attenuation; (ii) high reactogenicity and poor long-term immunogenicity (in humans); and (iii) transmissibility in nature. Previously, we reported that when the EMCV IRES was placed in lieu of the virus' subgenomic promoter, the resulting virus exhibited an attenuated phenotype and inability to replicate in mosquito cells. Here, we describe the use of IRES-based attenuation strategy for VEEV vaccine (subtypes ID and IE) construction and characterization. *In vitro*, VEEV/IRES vaccines produce small plaques and replicate to lower titers than the parental 68U201 (IE) or ZPC738 (ID) viruses. Mice injected subcutaneously with 1x10^5 pfu of VEEV/IRES show no signs of illness or changes in weight, produce neutralizing antibodies and are fully protected against their respective wild-type lethal challenge. Moreover, VEEV/IRES viruses are unable to propagate in C6/36 cells, implying that these viruses would be unable to be transmitted by mosquitoes in nature. These results, as well as those obtained from studies on chikungunya and eastern equine encephalitis viruses, demonstrate that the IRES-based method of alphavirus vaccine generation provides a predictable method for alphavirus attenuation while maintaining a host-restricted range of replication.

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HANTAVIRUS-DAF/CD55 ENGAGEMENT INITIATES RHOGTPASE ACTIVITY AND PARACELLULAR PERMEABILITY IN EPITHELIAL CELLS

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Pathogenic Hantaviruses cause hemorrhagic fever with renal syndrome (HFRS) and hantavirus cardiopulmonary syndrome (HCPS). Hantaviruses have profound tropism for microvascular endothelial cells, and capillary leak at sites of infection is important in pathogenesis. Hantaviruses are hypothesized to target the decay accelerating factor (DAF/CD55), a ubiquitous molecule expressed on the apical surface of polarized epithelia, as a co-receptor for accessing $\alpha_v\beta_3$ integrin, the entry receptor, expressed basolaterally. Initial engagement of DAF by UV-killed Sin Nombre virus results in upregulation of GTP-bound Rho GTPases (Rac1, Cdc42, RhoA), which is measured by confocal microscopy and G-LISA assays. The Rac1 inhibitor, NSC23766 and a novel Cdc42 inhibitor, ML 141 are used to establish the role of signal cross-talk among Rac1, Cdc42, and RhoA, during the induction of paracellular permeability in epithelial cells. These results are important for understanding the pathogenesis of hantavirus disease.

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EVIDENCE OF OUTDOOR BLOOD FEEDING IN THE HIGHLAND *ANOPHELES* OF WESTERN KENYA: A NEW CHALLENGE FOR MALARIA CONTROL?

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Indoor residual spraying (IRS) and long-lasting insecticide treated nets (LLINs) are core elements in most malaria control programmes. The effectiveness of these methods relies on the assumption that the majority of *Anopheles* vectors feed indoors and at times when people would be under a net. It is therefore vital to establish whether this assumption is true and determine the time and location that these mosquitoes bite. In addition to divergent behaviors, vector control methods may be undermined by development of insecticide resistance which will affect the success of both IRS and LLINs and may lead to the persistence of malaria transmission. This study aimed to compare the proportion and age of vector species biting at different hours of the night inside and outside houses in an area of seasonal low malaria transmission in Rachuonyo South district, Nyanza Province, western Kenya. This study also aimed to calculate the risk of infected bites with respect to the time that local residents entered their houses and used LLINs. The study took place between June 2011 and July 2012. Collections occurred each month for 6 nights, in 24 houses per night which were randomly allocated to indoor or outdoor trapping. CDC light-traps were hung adjacent to occupied LLINs, located either within houses or in rain shelters set outdoors. Hourly collections were made between 17:30 and 22:30 and then 05:30-06:30 the next morning. Questionnaires were used to capture the time that the local population entered houses and used LLINs. Seasonal fluctuations in vector species were recorded and more *Anopheles* were caught in outdoor traps. The morphological identification of vectors was confirmed by rDNA and mtDNA sequencing and exposure to each vector is discussed. There was evidence that the local population were at risk of infected bites both outdoors and indoors at a time when LLINs were not protecting a sizeable proportion of the population. Exposure to such bites may be responsible for maintaining transmission in the area. This work provides data on vector dynamics that can inform future malaria control programmes.

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EVALUATION OF AN ATTRACTIVE LETHAL OVITRAP (ALOT) AGAINST *AEDES AEGYPTI* FOR DENGUE CONTROL IN IQUITOS, PERU

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Dengue, the most important mosquito-borne virus world-wide, is primarily transmitted by the container-inhabiting mosquito *Aedes aegypti*. Without a vaccine to prevent new human infections, vector control remains the only method of preventing transmission. Female mosquitoes acquire the virus by taking blood from an infected human, then use a variety of cues to identify potential oviposition sites, where they must deposit eggs before taking another bloodmeal. We hypothesize that by specifically targeting older gravid females, we can most efficiently reduce dengue virus transmission. Toward that end, we have worked to develop an effective lethal ovitrap (Attractive Lethal OviTrap = ALOT) for *Ae. aegypti* control, with concentration on identification of oviposition attractants and stimulants. Starting in June 2011, we tested the ALOT in a large scale field trial in Iquitos, Peru, where dengue is endemic. The study design was a prospective nonrandomized controlled trial in two cohorts of Iquitos residents from two comparable city neighborhoods each of 2500 houses selected as either intervention or control zones. Traps were placed in houses at a density of ~3 per residence, with ~85% participation in the intervention area. Local ministry of health fumigation to control adult mosquitoes was ongoing in both areas during the study. Entomological indices were monitored in participating households at 3 month intervals, and individuals were monitored serologically, both through a longitudinal survey (at months 0, 12, 18) and through 3X weekly febrile surveillance. Nine months into the trial, dengue incidence as measured by febrile surveillance was 78% lower (0.3% vs. 1.34%) in the intervention area compared to the control area ($p<0.0001$). Confirmation of these results through separate longitudinal surveillance is pending. We also observed a difference in adult mosquito indices of approximately 50% (e.g. 65 to 30 females/100 houses) between the two areas. These preliminary results suggest that area-wide application with the ALOT could significantly lower dengue transmission.

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CHANGING BEHAVIORAL PATTERNS OF ARBOVIRAL VECTOR *AEDES AFRICANUS*: A CONCERN FOR EMERGING AND REEMERGING DISEASES

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Aedes (Stegomyia) africanus Theobald is a key arboviral vector to humans. It is a highly competent vector for several viruses that cause hemorrhagic fevers including; Yellow fever (YF), Rift Valley fever, dengue fever, and other arboviral vectors, most of which are widely distributed in Africa. There are no vaccines for these arboviral diseases except YF which has a safe and effective vaccine, yet YF outbreaks are still reported in Africa. Vector control is therefore crucial yet little is known about the biology of Ae africanus to enable effective vector targeted control and management of hemorrhagic fevers. Aedes africanus has specific behavioral preferences, some of which facilitate their spread within the rapidly changing landscape of Africa. *Ae. africanus* is reportedly confined to forests. However, it was implicated in the peridomestic transmission of YF to humans during the 1987 Nigerian YF epidemic. This study aims to understand the behavior of *Ae. africanus*. Bamboo pots containing water were placed on a 120 feet steel tower in Zika forest, Entebbe. The pots were placed at ground level, platforms at 20, 40, 60, 80, 100 feet above the ground and at shaded spots of the encroached forest buffer zone. Immature samples were collected weekly, reared to maturity and morphologically identified. After 32 weeks, a total of 734 mosquitoes were collected inside the forest, 89% of which were collected at 60 feet and below. A total of 642 Ae. africanus mosquitoes were collected at 200 and 400 feet from the forest boundary. These results indicate that *Ae. africanus* prefers to oviposit at levels below the tree canopy. These are shaded areas in the forest and heights at which the host, primates, are found. There is also a tendency for a change from a sylvatic (forest confinement) to a peridomestic behavior. This is probably due to the increased human activities in the forest buffer zone. Encroachment on the forest buffer zone must be strongly discouraged given previous isolations of several arboviruses from the forest, most of which have been isolated in *Ae. africanus*.

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SUMILARV 0.5G, A PROMISING INSECTICIDE FOR THE CONTROL OF *ANOPHELES GAMBIAE* S.L.?

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Malaria vector control with long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) has resulted in a significant decline of malaria in Africa. However, recent reports have shown both behavioural avoidance and physiological resistance to insecticides by vectors. Thus, the need to explore and integrate other tools for malaria vector control. Larval source management is a proven tool for malaria vector control. Difficulties with larviciding are (1) interventions are based at targeting all aquatic habitats and (2) current larvicides have short residual activity requiring weekly application which necessitate large labour and large amounts of larvicides which is not cost effective especially in Africa. Thus the need to evaluate persistent larvicides and study sound application strategies of the persistent larvicides. Sumilarv is a persistent larvicide with great potential for mosquito control. Bioassays showed that Sumilarv was ten times more effective that microbials for *Anopheles* control. Sumilarv application at 0.05 ppm a.i. resulted in 80% emergence inhibition for up to 6 weeks under standardized field conditions. Evaluations of the optimum dose of Sumilarv identified in the standardized field tests are on-going in an area of focal malaria transmission in Western Kenya. Preliminary results from the first three months of field testing indicate that the three week application results on average in 71% emergence inhibition of malaria vectors from treated sites. The use of residual larvicides has a risk of vector production from untreated habitats created or filled with water after larvicide application. Intensive monitoring and sampling of sites is ongoing to estimate this risk in the dry and rainy seasons.

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DECLINE IN FREQUENCY OF THE 2LA CHROMOSOMAL INVERSION IN AN *ANOPHELES GAMBIAE* S.S. POPULATION WITH INCREASING USE OF INSECTICIDE TREATED BED NETS IN WESTERN KENYA

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The 2La chromosomal inversion is polymorphic in populations of *Anopheles gambiae* s.s. and has been positively associated with indoor resting behavior. Although the genotype/phenotype relationship is not precisely known, it is likely adaptive for microclimatic conditions related to humidity. Specifically, reports from West Africa indicate that the 2La arrangement is more common in mosquitoes found resting indoors where a nocturnal saturation deficit exists. Ownership of insecticide treated bed nets (ITNs) has risen rapidly in western Kenya in the last decade, with subsequent declines in malaria transmission and malaria-related mortality and altered vector population genetic structure, with an increase to fixation of the East African kdr allele in *An. gambiae* s.s. Our study focused on the frequency of the 2La chromosomal inversion of *An. gambiae* s.s. in that setting. Adult *An. gambiae* mosquitoes were sampled from 1996 to 2011 in Asembo, an area with high ITN coverage since 1999, and from the adjacent community of Seme, where ITN ownership was <5% in 1999 but increased to over 60% by 2006. The 2La analysis was done using a PCR assay with primers designed for 2La and 2La+ proximal breakpoints and visualization of amplicons by electrophoresis on agarose gels. In Asembo, the frequency of the 2La chromosomal inversion declined from 93% in 1996 to 15% in 2005 and remained low through 2011(21%). Similarly in Seme, the frequency declined from 55% in 2000 to 19% in 2005 and remained low in 2008 (17%). These results suggest that high coverage of ITNs may have selected for the 2La+ chromosomal arrangement in *An. gambiae* s.s, a genotype not associated with indoor resting. A possible explanation is that ITNs are effective against indoor resting *An. gambiae* s.s., which are more likely to have the 2La inversion karyotype. Further studies are proposed to determine if populations with the 2La+ karyotype successfully avoid ITNs, and are responsible for maintaining residual malaria transmission in areas with high ITN coverage.

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AN EXAMPLE FROM ZAMBIA OF USING NOVEL APPROACHES TO MONITORING AND MANAGE INSECTICIDE RESISTANCE FOR EFFECTIVE VECTOR CONTROL

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Increased coverage with insecticide treated nets (ITNs) and indoor residual spraying (IRS) with DDT and pyrethroids, have led to impressive decreases in malaria transmission in Zambia. However, the detection of high levels of insecticide resistance in both *Anopheles gambiae* and *An. funestus* is a serious risk to vector control efforts. In 2011 an Insecticide Resistance Management Technical Working Group was established to develop a plan to sustain current control levels and conserve insecticides for malaria control in the country. Here we report on results from bioassays and molecular analysis of resistance mechanisms for two regions, the Copperbelt and Eastern Provinces of Zambia. A high prevalence of resistance to deltamethrin (27% Mortality), permethrin (34%M), etofenprox (5%M) and DDT (6%M) was detected in *An. gambiae* from the Copperbelt; the same populations were susceptible to bendiocarb and malathion. Resistance to the pyrethroids and DDT was due to kdr (the 1014F mutation is fixed in this population) and over expression of several p450's including *CYP6Z3* and *CYP6M3*. *An. funestus* from Eastern Province also exhibited resistance to diagnostic doses of deltamethrin (45% M), permethrin (81.5% M), etofenprox (18%M) and bendiocarb (77% M), but was susceptible to DDT. This population has elevated *CYP6P9a, CYP6Z1* and *CYP6M3*. A more susceptible population of *An.funestus* was found in the Copperbelt and only had elevated *CYP6M3*. As well as a different resistance profile in these regions the collections indicated very different malaria vector species abundance patterns that will impact vector control decisions. The impact of this information has allowed the Zambian malaria control programme to move away from ineffective insecticides used in the Copperbelt (DDT) and Eastern (etofenprox) to effective insecticides and to put an insecticide resistance management programme in place with the aim of prolonging the successes already gained. We examine the entomological M&E in Zambia and how lessons learnt here can be applied to other vector control programmes in the region.

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A LONG-LASTING *BACILLUS SPHAERICUS* (BS) AND *BACILLUS THURIGIENSIS VAR ISRAELENSIS* (BTI) FOR CONTROLLING MALARIA VECTORS: TRIALS FROM KENYA

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Bio-larvicides are alternatives for larval mosquito control as they are benign to the environment instead of synthetic insecticides. However, the currently available bio-larvicide formulations have a short effective duration, and consequently larval control incurs a high operation expense due to requirement for frequent re-treatment of larval habitats. Therefore, formulation of biological larvicides that has long-lasting effects is highly desired. A recently developed fourStarTM Single Brood Granules (SBG)

of Bacillus thuringiensis israelenis (Bti) was evaluated under semi-natural and natural conditions to test its effectiveness in reducing mosquito population in western Kenya. This formulation is designed to be effective against mosquito larvae for up to 6 months. In semi-natural habitats containing soil and rain water, second-instar larvae of *Anopheles gambiae* were introduced and FourStarTM Bti granules dissolved in rain water with appropriate concentrations were added. The number of pupae produced from the larvae was recorded daily as the outcome. Formulation was also tested in natural productive habitats. Formulation was then tested for its efficiency to reduce mosquito population during the transmission season, when it is applied earlier in sentinel sites. Larval control was undertaken in field trials in three sites and with three other sites taken as control. We found 100% mortality rate within 48 hrs after introduction of 2nd instars larvae in semi-natural habitats. The Bs/Bti formulation killed larval mosquitoes for 6months. Formulation killed larvae for 5 months in natural habitats despite the effects of rain. In larval control field trials the formulation reduced density of mosquitoes in houses from between 60-80% in the intervention sites during the transmission season. Larval control has the potential to reduce the population of malaria mosquitoes. The Bs/Bti briquets present a promising biological formulation to use for larval control. This formulation is recommended to the National Malaria Control programme.

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RE-ASSESSMENT OF DENGUE NEUTRALIZING ANTIBODY AND VIREMIA TITERS IN DENGUE PATIENTS USING FCΓR-EXPRESSING CELLS

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One of the major obstacles in dengue vaccine development is the potential infection-enhancement activity induced by vaccination. Sub-neutralizing levels of antibody against dengue virus (DENV) is speculated to enhance infection, and play a central role in the pathogenesis of severe and life-threatening illness, dengue hemorrhagic fever (DHF). General understanding on the biological properties of antibody in protection against dengue infection is based on the titers determined by the use of FcγR-negative cells in conventional neutralizing antibody. Additionally, conventional viremia titration assays do not consider infectious immune complex which may be infectious only through FcγR. Using FcγR-expressing BHK cells and FcγR-negative BHK cells, we examined the infection-enhancement activity and neutralizing activity in serum samples from patients with secondary and primary DENV infection. Serum samples with low neutralizing activity demonstrated infection-enhancing activity and those with high neutralizing activity demonstrated low or no infection-enhancement activity in FcγR-expressing cells. Additionally, neutralizing activity to the infecting DENV serotype detected by using FcγR-negative was absent in FcγR-expressing cells. Higher levels of viremia were detected using FcγR-expressing cells as compared to FcγR-negative cells in serum samples obtained from patients and a dengue non-human primate (NHP) model during secondary dengue infection. The results suggest that DENV-antibody complexes which are incapable of infecting FcγR-negative cells retain infectivity in FcγR-expressing cells due to infection mechanism through FcγR. Our findings suggest that in comparison to FcγR-negative cells, FcγR-expressing cells may better reflect the biological properties of antibodies *in vivo*. In summary, we established an assay which possesses the ability to detect the sum of infection-enhancement and neutralizing activities. The newly developed assay provides a platform to define dengue virus infectivity and viremia titers in the presence of neutralizing and enhancing antibody activities and offer insights into the role of antibodies in protection in natural infection and vaccination.

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DISSECTING HUMAN ANTIBODY RESPONSES TO SILENT AND CLINICALLY-APPARENT DENGUE VIRUS INFECTION

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Dengue is the most significant vector-borne viral disease of humans. The dengue virus (DENV) complex consists of 4 serotypes. Following primary DENV infection people develop immunity to the infecting serotype, but remain susceptible to a second infection with a new serotype. Secondary DENV infections are more likely to result in severe disease than primary infections. Antibody dependent enhancement is proposed to explain this phenomenon. Using prospectively collected samples from a cohort of children from Colombo, Sri Lanka, we explored the quantity and quality of pre-infection antibodies in children who experienced secondary silent and apparent DENV infections. Quantity of DENV-specific IgG was determined by ELISA, and antibody quality was determined by performing virus neutralization or enhancement assays. Children who acquired secondary silent and apparent DENV infections had similar pre-existing DENV-specific IgG levels. However, children who acquired secondary silent DENV infections had pre-existing antibodies that were more broadly neutralizing than children who acquired secondary apparent DENV infections. In this presentation, we will also discuss the ability of pre-infection antibodies from silent and apparent cases to enhance DENV infection of Fc-receptor bearing cell lines and primary human cells. Together, our findings demonstrate how neutralization capacity and enhancement ability of pre-existing antibodies influences disease presentation in secondary dengue infections.

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LONGITUDINAL ANALYSIS OF THE LEVELS OF CROSS-REACTIVE ANTIBODIES RECOGNIZING THE FUSION LOOP OF DENGUE VIRUS AND CORRELATION WITH NEUTRALIZING ANTIBODY TITERS IN NICARAGUAN DENGUE CASES

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The envelope (E) protein of dengue virus (DENV) is the major target of neutralizing antibodies (Abs). Previous studies of human convalescent sera after DENV infection revealed that a significant proportion of anti-E Abs recognized the highly conserved fusion loop (FL) of domain II of E protein (FL Abs), whereas a minor proportion recognized domain III. The role of FL Abs in dengue pathogenesis remains unclear. In this study, we tested the hypothesis that cross-reactive FL Abs, though not contributing to the monotypic neutralization profile after primary DENV infection, may play a role in protection against heterologous serotypes after secondary DENV infection. A quantitative virion-capture ELISA was established by using known concentrations of a human anti-E monoclonal Ab as a standard to measure the concentration of anti-E Abs, [anti-E Abs], in sera of dengue patients from Nicaragua. The proportion of FL Abs was determined by a previously described capture ELISA using virus-like particles, and the concentrations of FL Abs, [FL Abs], were calculated. Neutralization titers were determined using a flow cytometry-based neutralization assay with reporter viral particles of the different DENV serotypes. Analysis of sequentially collected serum samples (3M, 6M, 12M and 18M) from 10 cases of primary or secondary DENV infection revealed that [anti-E Abs] and [FL Abs] stabilized at 12 M after infection and were higher in secondary DENV infection cases than in primary infection cases. The [FL

Abs], while not correlated with neutralization titers in primary infection cases, increased as the neutralization titers against heterologous serotypes increased in secondary infection cases. These findings are being verified in sera from 26 additional secondary DENV infection cases. Our results demonstrate the kinetics of FL Abs over time after DENV infection and suggest that FL Abs might play a protective role against heterologous serotypes after secondary DENV infection.

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CORRELATION BETWEEN DENGUE VIRUS-SPECIFIC NEUTRALIZATION, SERUM AVIDITY AND ANTIBODY TITERS IN PRIMARY AND SECONDARY DENV-3 NATURAL HUMAN INFECTIONS

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The 4 serotypes of dengue virus (DENV1-4) infect ~100 million people annually. While heterotypic secondary (2) DENV infection has been associated with severe disease, the vast majority of 2 infections are mild or asymptomatic, suggesting protective cross-reactive immunity in addition to long-lasting homotypic immunity. The mechanism of antibody (Ab)-mediated protection is not well defined. We are analyzing DENV-specific neutralization titer, IgG avidity and Ab titer in well-characterized serum samples from a pediatric dengue hospital-based study in Managua, Nicaragua. In 2010, 130 DENV-positive cases were enrolled (primary (1) n=75; 2 n=55), with DENV3 as the dominant serotype (83.1%). The 50% neutralization titer (NT_{50}) was measured by flow cytometry. Avidity and Ab titer were measured by a modified ELISA with urea washes and by Inhibition ELISA, respectively. We observed a significant increase in avidity vs. DENV3 between the convalescent and 3-month (3m) timepoints (% IgG bound = 45.8 vs. 82.6, p<0.0001) in 1 infections, reflecting affinity maturation. We also noted a significant increase in avidity between the acute and convalescent phase in 2 infections (69.2% vs 79.7, p=0.0015), without further increase over time (3-6m), attributable to newly formed Ab against the current infecting serotype. The NT_{50} peaked at convalescence in both 1 and 2 cases, with significantly higher titer detected in 2 cases (5284 $\pm$ 683 vs. 11476 $\pm$ 1183, p<0.0001). In the convalescent phase and 3m after 1 infection, neither DENV3-specific avidity nor DENV-specific Ab titer correlated with DENV3-specific NT_{50}, implying that either innate immune and/or naïve T cell responses and/or low-avidity Abs control 1 infections. In acute 2 infections, we observed a correlation between avidity and NT_{50} vs. DENV3 (Spearman r=0.50, p=0.002) and a correlation with DENV-specific Ab titers (Spearman r=0.61, p<0.0001), most likely reflecting an expansion of cross-reactive DENV-specific memory B cells formed during the previous infection. We are currently processing these samples against DENV2, the most likely 1 infecting serotype, to confirm this hypothesis. Lastly, we find that at the 3m timepoint, DENV3-specific avidity correlates positively with DENV3-specific NT_{50} (Spearman r=0.49, p=0.0015). A better understanding of the protective immune response in natural infections is critical for the development of safe and effective vaccines.

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MAPPING ENHANCING ANTIBODIES PRODUCED BY THE HUMAN IMMUNE RESPONSE AFTER PRIMARY DENGUE VIRUS INFECTIONS

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Dengue virus (DENV) is a mosquito-borne flavivirus of global significance. DENV exists as four serotypes, named DENV1 through DENV4. Following a primary infection, individuals produce a mixture of type-specific and cross-reactive antibodies (Abs). Pre-existing immunity is sufficient to protect against re-infection with the same serotype, but may enhance infection and increase disease severity during a secondary infection with one of the other three DENV serotypes. A leading theory to explain the higher frequency of severe disease is the antibody-enhancement (ADE) theory, where a fraction of pre-existing DENV-specific Abs are thought to bind viral particles and aid infection of host cells through Fcγ receptors. Due to the complexity of the human humoral immune response, the enhancing anti-DENV Abs within human polyclonal sera have not been well characterized. Previously, Abs in DENV-immune human sera were fractionated using DENV virions, and the role of specific antibody populations in DENV enhancement was investigated in cell culture and in the AG129 mouse model of DENV infection and disease. We demonstrated that people exposed to primary DENV infections have serotype-specific and serotype cross-reactive populations of circulating Abs. The serotype-specific Abs were responsible for neutralization of the homologous serotype, whereas the serotype cross-reactive Abs were responsible for ADE of heterologous serotypes. The ability of the serotype cross-reactive Abs to enhance DENV was observed both *in vitro* and *in vivo*. Further studies were then performed to identify the antigens and epitopes engaged by enhancing Abs in human serum by fractionating DENV-immune sera using recombinant viral proteins and assaying the depleted sera in *in vitro* ADE assays and in the AG129 mouse model. Our studies demonstrate that enhancing Abs in DENV-immune sera recognize epitopes on E protein as well as prM. Further studies are in progress to quantify the relative contribution of Abs against different antigens to ADE and to map specific epitopes responsible for ADE.

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ANTIBODY RESPONSES TO THE DENGUE VIRUS PROTEOME DURING SEASONAL OUTBREAKS OF INFECTION

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Dengue is a mosquito-borne infection caused by four distinct serotypes of dengue virus, each appearing cyclically in the tropics and subtropics along the equator. The viral proteome is comprised of capsid, membrane, envelope and the non-structural (NS) proteins NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5. Though each of these proteins synthesized during infection are potential targets of host defenses, current knowledge of the immune response to the DENV proteome is limited. Here we describe a protein microarray approach for measuring antibody responses to the complete viral proteome of dengue virus serotypes 1-4. Using this microarray, we examined humoral immunity to dengue occurring during seasonal outbreaks in Puerto Rico, and identified unique immunological profiles resulting from pediatric and adult infections. Our results demonstrate discriminating details concerning the nature of antibody responses to dengue virus at the proteomic level and suggest the usefulness of this information for vaccine development.

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CONSIDERING THE ROLE OF ANTIBODY IN DENGUE VIRUS CLEARANCE: DATA ANALYSIS AND MODELLING

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Antibodies in dengue infection are thought to play a critical role in controlling infection, but also may enhance viral replication in secondary infection via the phenomenon of antibody-dependent enhancement. Here, we consider mainly the former role, using sequentially sampled measurements of virus and antibody titres (IgM, IgG, anti-E IgG and anti-D3 IgG) from patients hospitalised with dengue infection in Vietnam. Analysis of such data is not straightforward, due to differences in timing of measurements relative to virus peak and symptoms onset, however using multiple sequential measurements from throughout natural infections is an excellent way to consider the interaction between virus and the immune response. In addition to descriptive statistical analyses, we fitted a mechanistic mathematical model of dengue pathogenesis within the human host to these data to investigate whether the observed kinetics were compatible with antibody playing the dominant role in controlling viral replication. A model variant which assumes clearance of virus or infected cells is proportional to overall IgM titres is able to fit data from both primary and secondary infections, and the same model with clearance proportional to overall IgG titres is able to fit data from secondary infections. However, this fit relies on variation in how much measured antibody is useful, and in some individuals there are issues in timing of virus peaks and antibody increase. A model for secondary infections in which viral clearance rates were proportional to anti-E IgG titres gives, in most cases, the best model fit, overcoming some of these issues. We will also present extensions to this work to include mechanistically the interactions between the different antibody measures. Interestingly, for all model fits, we estimate that the level of antibody required to control viral replication is low, and over an order of magnitude below the peak titres reached by the time infection is cleared. In our presentation we will consider the implications of this result for measurement of antibody kinetics.

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INNOVATIVE IMMUNOLOGICAL ASSAYS FOR DIAGNOSIS OF *SCHISTOSOMA MANSONI* FOR CLINICAL ACUTE AND/OR CHRONIC FORMS

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Control constraints of schistosomiasis include the lack of diagnostic methods with high sensitivity. We initiated a prospective study in southeast Brazil in order to develop sensitive diagnostic methods for *Schistosoma mansoni* infection. Residents on 3 endemic areas in Minas Gerais state, together with 84 travelers infected in a freshwater pool on a country house located on a new focus of the disease, participate of this survey. Sera samples from all those patients are used for the standardization of innovative methods for schistosomiasis mansoni. Comparisons are performed with the presence of eggs in faecal samples, IgG antibody titers, presence of eggs in liver after biopsy, encephalomyelitis by magnetic resonance imaging, and/or clinical symptoms. The first assay using schistosomula antigen is capable of properly diagnosing all the 84 travelers with clinical acute form as soon as 10 days post-infection, including patients with severe hepatic form and encephalomyelitis. Two other assays using egg and adult worm antigens are capable of detecting more than 95% of positive cases from chronic and low parasite load patients (1-36epg). A forth method called Immunomagnetic separation (IMS) was developed in order to concentrate sera samples. We used several antigens, in separate, for IgG titers detection, as purified glycoprotein Circulating Cathodic Antigen (CCA), CCA recombinant protein (CCAr), and five different types of peptides (10 amino acids each) of CCA. Data showed that IMS is superior to ELISA ($p=0.001$) since it is capable of detecting a higher number of positive patients. The purified CCA was not a good candidate due to its susceptibility for cross reaction. On the contrary, peptides and especially CCAr, are excellent tools for the differential diagnosis with 100% of sensitivity. Furthermore, IMS method was standardized for a direct detection of CCA in sera. For that matter, monoclonal antibodies against the protein portion of the native CCA (MAb-CCA) were produced. Using only 0.05ml of concentrated sera, we were able to detect 100% of chronic patients and 98% of patients with acute form of the disease. Finally, a last methodology were developed, a qualitative method using magnetic beads and CCA-MAb conjugated to Alexa Fluor for the direct visualization of fluorescent CCA in sera samples. A double-blinded study showed that 3 slides of each sample are sufficient to achieve a sensitivity of 98% and a specificity of 95%.

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DIAGNOSTIC APPROACHES FOR PEDIATRIC TUBERCULOSIS AMONG HIV-INFECTED AND HIV-UNINFECTED CHILDREN IN PERU

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Children with pulmonary tuberculosis (PTB) usually present with paucibacillary disease and without sputum, and HIV co-infection may further complicate diagnostic testing. We evaluated HIV-infected Peruvian children with suspected PTB with a series of culture and PCR-based techniques, and compared results from these subjects with similar results from HIV-infected controls and from HIV-negative cases and controls. TB culture and a heminested IS 6110 polymerase chain reaction (PCR) assay were performed on specimens from children with symptoms of PTB and well controls. Two specimens of each type (gastric aspirates [GA], nasopharyngeal aspirates [NPA], and stools) from each case were examined by 1) auramine smear, 2) broth culture by Microscopic-Observation Drug-Susceptibility (MODS) technique, 3) standard culture on Lowenstein Jensen (LJ) agar, and 4) PCR. Specimens from controls included one NPA and 2 stools. The study included 209 HIV-negative cases, 81 HIV-positive cases, 200 HIV-negative controls, and 35 HIV-positive controls. Overall, 22 HIV-negative case subjects (10%) had at least one positive TB culture. In contrast, TB was only isolated from one HIV-positive case (1.2%), from both GA specimens only ($p<0.01$). In contrast to the difference in TB isolation between HIV-positive and HIV-negative cases, the proportions of subjects in these groups with at least one positive PCR result were similar, and both case groups had more positive PCR results than the HIV-negative controls ($p<0.001$). Rates of PCR positive specimens were similar for HIV-positive cases and controls. In contrast to reports from Africa, TB recovery from HIV-positive patients with suspected PTB in our Peruvian pediatric population is less common. In spite of the differences in culture-based MTB recovery, HIV-positive cases had similar rates of PCR-positive specimens as compared to HIV-negative case subjects. These PCR-positive, culture-negative specimens may reflect paucibacillary disease, or in HIV-positive controls they may indicate latent or subclinical infection.

1401

PEPTIDE YY, GHRELIN, LEPTIN AND IL-10 AS MEDIATORS OF APPETITE AND RESPONSE TO TREATMENT IN PERUVIAN ADULTS WITH TUBERCULOSIS

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Cachexia is one of the sentinel symptoms of pulmonary tuberculosis (TB). TB causes an inflammatory response that leads to alterations of appetite hormones affecting appetite and satiety. Yet the relationship of TB disease severity and appetite hormone levels has not been well studied, despite its potential utility as an indication of treatment failure. 23 adult patients with sputum positive TB were evaluated on days 0, 14, and 28 days of treatment by Simplified Nutritional Appetite Questionnaire (SNAQ), body mass index (BMI), and appetite and inflammatory markers. Peptide YY, ghrelin, leptin, and IL-10 levels were determined using Luminex and ELISA kits. We also administered a questionnaire to qualitatively determine appetite. Appetite questionnaire results trended towards a gain in appetite with treatment, displaying a significant difference between day 0 vs.14 (ρ=0.004) and day 28 (ρ=0.0095). Peptide YY levels dropped 14.6% by Day 14 of treatment (not significant), while ghrelin levels dropped 54% by Day 14 (ρ<0.05). Leptin levels increased 67.36% by day 28 of treatment (ρ<0.05), and the anti-inflammatory cytokine IL-10 decreased 12.5% by Day 14 (not significant). Subjective appetite improved with treatment as early as day 14, while BMI was slower to respond and still had not increased significantly by day 30. Delayed recovery of weight gain suggests that the increase in leptin is secondary to TB infection. Wasting in TB patients may partly be mediated by upregulation of anorexigenic PYY with resulting appetite suppression. Decrease in IL-10 levels may indicate intact immunity with normal response to treatment. Deviation from improving appetite status, clinical factors and appetite hormone levels may be used to detect treatment failure in cases such as multi-drug-resistant TB. While loss of appetite is a well-known symptom of TB, little work has been done in utilizing measurements of appetite in the characterization of the disease, and this work suggests that it may be a useful indicator of treatment success.

1402

BRUCELLOSIS AMONG HOSPITALIZED FEBRILE PATIENTS IN NORTHERN TANZANIA

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Brucellosis is an important cause of zoonotic disease worldwide. However, non-specific clinical features, low clinical suspicion, and lack of access to adequate diagnostic services result in brucellosis being underdiagnosed and untreated in many low-resource countries. Human clinical data are scarce in sub-Saharan Africa. Acute and convalescent serum samples were collected from febrile inpatients admitted to two hospitals in Moshi, Tanzania serving a catchment area dominated by smallholder farming communities. Confirmed brucellosis was defined as a positive blood culture for *Brucella* spp or a ≥4-fold increase in microagglutination test (MAT) titer, and probable brucellosis was defined as a single reciprocal titer ≥160. A total of 870 patients were enrolled in the study, 403 (46.3%) adults and adolescents and 467 (53.7%) infants and children. Of 455 participants with paired sera tested for brucellosis, 16 (3.5%) met criteria for confirmed brucellosis. Of 830 participants with ≥1 serum sample, 4 (0.5%) met criteria for probable brucellosis. Five (31.3%) of the participants with confirmed brucellosis were female. The median (range) age of participants with confirmed brucellosis was 28.4 (1.1, 68.5) years. Brucellosis was associated with increased median age (p = 0.024), leukopenia (odds ratio [OR] 7.8, p = 0.005), thrombocytopenia (OR 3.9, p = 0.018), and evidence of other zoonoses (OR 3.2, p = 0.026). There was no association between brucellosis and rural residence, hepato- or splenomegaly, lymphadenopathy, anemia, pleural effusion, or HIV. Brucellosis was never diagnosed clinically. Although all participants with brucellosis received antibacterials or antimalarials in the hospital, none received standard brucellosis treatment. Brucellosis is an underdiagnosed and untreated cause of febrile disease among hospitalized adult and pediatric patients in northern Tanzania. Increased clinician awareness, access to reliable diagnostic tests, and additional research on risk factors are needed to identify, appropriately manage, and prevent brucellosis in this area.

1403

INFECTIOUS DISEASES ARE A LARGER CONTRIBUTOR THAN OBSTETRIC CAUSES TO MATERNAL MORTALITY IN RURAL WESTERN KENYA

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Improving maternal health is a high priority for the United Nations' development agenda where it is targeted as the fifth Millennium Development Goal. In Kenya, the maternal mortality ratio remains high, at 488 per 100,000 live births per the 2008/09 Demographic Health Survey.

It is commonly assumed that maternal deaths are primarily a result of direct obstetric complications that occur around the time of childbirth. We conducted descriptive analyses of data from a Health and Demographic Surveillance System encompassing a population of approximately 220,000 individuals in rural western Kenya, an area that bears a disproportionate share of infectious diseases. Standard WHO methodology for verbal autopsy (VA) was implemented to determine contributors to maternal mortality (defined as the death of a woman while pregnant or within 42 days of the termination of pregnancy). The maternal mortality ratio for the six year period between 2003 and 2008 was 740 per 100,000 live births, with no evidence for a linear trend over time. Of 249 maternal deaths, one-third (n=85) were due to directly ascribed causes, predominantly by postpartum hemorrhage (n=22), complications from abortion/miscarriage (n=14), and puerperal sepsis (n=13). However, the majority of maternal deaths (n=164) were classified through VA as deaths from infectious diseases, predominantly from HIV (n=74), malaria (n=22) and TB (n=16). While the impact of HIV on maternal mortality has been previously recognized, in this area with high levels of malaria transmission, malaria was also a significant factor among deaths of pregnant or recently delivered women (65 maternal deaths associated with malaria per 100,000 live births). This was equal to the number of directly attributed obstetrical deaths due to documented postpartum hemorrhage. These data add to our awareness of the relationship between infectious diseases and poor maternal outcomes in Africa. Our data suggest that improved access to, and increased uptake of, emergency obstetric care, as well as preventive measures against HIV, malaria and TB among all women of childbearing age, will result in measurable impact on maternal health outcomes.

1404

SCABIES COMMUNITY PREVALENCE AND MASS TREATMENT IN TWO FIJIAN VILLAGES

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Scabies is a major public health problem with complications caused by bacterial secondary infection. A community mass treatment study in two Fijian villages was undertaken to compare the efficacy and tolerability of topical benzyl benzoate and oral ivermectin. Two research sites with approximately 600 inhabitants each were chosen, and study participants enrolled, completed questionnaires and examined to assess for scabies. In one village participants received benzyl benzoate and in the other either oral ivermectin or, for children under 2 years, permethrin cream. At follow-up, participants were re-examined and possible adverse events documented. Pre and post-treatment questionnaires included questions regarding history, itch, adverse events and satisfaction with treatment. Although ethnic and age demographics were similar in the two villages, scabies prevalence rates differed significantly, 38% and 24%. Prevalences in both villages were particularly high in children, with superinfection of scabies lesions common. Only 43% of those treated returned for follow-up overall. The scabies prevalence rate in those who returned for follow-up dropped from 37.9% to 19.9% after treatment with benzyl benzoate, compared to 23.7% and 9.5% following ivermectin treatment. Thus scabies prevalence was reduced by 53% following therapy with benzyl benzoate, and by 52% in those who received ivermectin. People treated with benzyl benzoate more commonly reported initial worsening of itch and of pre-existing dermatologic conditions after application than those treated with ivermectin. No serious side effects occurred with either treatment, and patient satisfaction did not differ between the treatments. In conclusion, mass treatment with oral or topical therapy in a village setting with high prevalence of scabies is feasible. Despite the difficulties in assessing ongoing active scabies infestation when the papules persist, a reduction in scabies prevalence of 53% and 52% was recorded.

1405

FACTORS INFLUENCING ATTENDANCE AT TREATMENT AND PREVENTION CLINICS BY PATIENTS WITH PODOCONIOSIS IN SOUTHERN ETHIOPIA: A QUALITATIVE STUDY

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Podoconiosis is a lymphoedema of non-infectious cause which results in long-term ill health in affected individuals. Simple, effective treatment is available in certain parts of Ethiopia, but anecdotally, not all patients continue collecting treatment supplies from clinic sites once started. We used qualitative techniques to explore factors affecting continued collection of treatment supplies from outreach clinics of a non-government organization in southern Ethiopia. A cross-sectional qualitative study was conducted in four clinic sites through unstructured in-depth individual interviews, key informant interviews and focus group discussions with the involvement of 88 study subjects.Sub-optimal continuation with clinic visits is common among podoconiosis patients. The reasons were: remoteness from the clinic sites, unrealistic expectation of 'special' aid, worry about increasing stigma, illness, misconceptions about treatment, and being too busy.Several of these factors are remediable through community and individual information and education. Appropriate routes to deliver this information must be identified. Certain factors (such as distance to clinic sites and stigma) require substantial expansion of services or liaison with village-level government health services.

1406

PREVENTION OF TUNGIASIS AND TUNGIASIS-ASSOCIATED MORBIDITY USING A HERBAL REPELLENT: A RANDOMIZED CONTROLLED FIELD STUDY IN RURAL MADAGASCAR

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Tungiasis (sand flea disease) is a neglected tropical disease. It is endemic in many resource poor populations in South America, the Caribbean and in sub-Saharan Africa and is associated with significant morbidity. Since there is no effective drug treatment, prophylaxis is the only means to prevent sand flea disease. In a randomized, controlled field study in rural Madagascar, two preventive measures were compared: the twice-daily application of Zanzarin (a repellent based on coconut oil) on the feet and the free distribution of closed shoes. A control group was left without any intervention. Over a period of 10 weeks, study participants were examined every two weeks and the number of newly penetrated sand fleas, the total number of lesions, the proportion of different developmental stages, and tungiasis-associated morbidity were determined quantitatively. Compared to the control group, the total number of penetrated sand fleas decreased only by 5% after the distribution of shoes. The regular application of Zanzarin reduced the parasite load by 85%. In the shoe group, the median attack rate fell by 22%, in the Zanzarin group by 95%. The distribution of shoes reduced tungiasis-associated morbidity only marginally. The protective effect of shoes was related to the regularity with which shoes were worn. After 10 weeks of application of the repellent tungiasis-associated morbidity had disappeared almost completely. The study shows that twice-daily application of a repellent based on coconut oil provided an excellent protection against the development of sand flea disease. The free distribution of shoes had only a minimal protective effect, mainly because shoes were not worn regularly.

1407

IMPACT OF INTRODUCTION OF THE *HAEMOPHILUS INFLUENZAE* TYPE B CONJUGATE VACCINE INTO CHILDHOOD IMMUNIZATION ON MENINGITIS IN BANGLADESHI INFANTS

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Some Asian countries have been reluctant to adopt Hib vaccination because of uncertainty over disease burden. We assessed the impact of introduction of Hib conjugate vaccine into the Expanded Program on Immunization (EPI) in Bangladesh on purulent and laboratory confirmed *Haemophilus influenzae* meningitis. Within a well-defined catchment area around two surveillance hospitals in Dhaka, Bangladesh, we compared the incidence of Hib meningitis confirmed by culture, latex agglutination and polymerase chain reaction (PCR) assay among infants one year before and one year after introduction of Hib vaccine. We adjusted the incidence rate for the proportion of children who sought care at the surveillance hospitals. Among infants, the incidence of confirmed Hib meningitis decreased from 92 to 16 cases per 100 thousand within 1 year of vaccine introduction [Vaccine preventable incidence (VPI) =76; 95% CI: 18, 135/ 100 thousand]. The incidence of purulent meningitis decreased from 1659 to 1159 per 100 thousand [VPI=500; 95% CI: 203, 799/ 100 thousand]. During the same time period, there was no significant difference in the incidence of meningitis due to *Streptococcus pneumoniae*. Introduction of conjugate Hib vaccine into Bangladesh EPI markedly reduced the burden of Hib and purulent meningitis.

1408

IMMUNOGENICITY, SAFETY, DOSE AND SCHEDULE RESPONSE OF A MENINGOCOCCAL GROUP A CONJUGATE VACCINE IN INFANCY: A HOPE FOR ROUTINE IMMUNIZATION IN THE AFRICAN MENINGITIS BELT

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Meningitis epidemics remain a major plague in countries in the African meningitis belt, with group A meningococcus being the predominant causal agent. An affordable meningococcal group A conjugate vaccine was developed through the Meningitis Vaccine Project, and introduced at public health scale in 2010-11, using single dose mass campaigns among 1 to 29 year-olds in 6 out of 26 target countries of the meningitis belt, with extremely promising results. Roll-out in all countries is ongoing. To maintain population immunity level after initial campaigns, protection of new birth cohorts should be achieved early in life. We conducted a dose ranging study of the newly developed MenA conjugate vaccine in infants to evaluate the safety and immunogenicity of three different doses administered in a two dose schedule at 14 weeks and 9 months, or in one dose schedules at 9 or 12 months concomitantly with the EPI vaccines. Starting in 2008, 1198 infants were recruited in the Kassena Nankana districts of Northern Ghana and followed up till 2012. Results confirmed noninferiority of the alternate dosages to the licensed dosage. No significant interferences with co-administered EPI vaccines were found. The proportions of subjects with seroconversion at Day 28 post 9 months vaccination were high and similar in all MenA vaccine groups (1 or 2 doses regimens), but the magnitude of the responses was higher in subjects previously primed with MenA vaccine (2 doses regimens *vs.* 1 dose regimen), nonetheless administration of a single dose at 9 months of age induced a high immune response. No significant safety concerns were identified. The majority of adverse events were due to infections consistent with background morbidity in the area. Sustainable protection from MenA disease among new birth cohorts could be achieved through immunization starting in late infancy at 9 months. This could be a powerful strategy for sub-Saharan countries, leveraging on vaccine herd protection effect, preventing overcrowding early infancy schedules, and allowing paired administration of the MenA with that of the measles vaccine.

1409

PERSISTENT, WIDESPREAD OUTBREAK OF TYPHOID FEVER ASSOCIATED WITH INTESTINAL PERFORATIONS - BUNDIBUGYO AND KASESE DISTRICTS, UGANDA, 2009-2011

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Salmonella enterica serovar Typhi causes approximately 22 million typhoid fever infections worldwide each year; among these, 1-3% of patients develop intestinal perforation (IP). In 2008, an outbreak of typhoid fever with a high rate of IP was reported in Kasese, a rural district in western Uganda. A 2009 investigation of this outbreak identified 577 cases through July 15, 2009; 249 had IP. A high rate of IP was sustained in Kasese through 2011 and the neighboring district of Bundibugyo reported a typhoid fever outbreak in August 2011. We gathered information about cases through enhanced surveillance and hospital and district health office (DHO) records. A suspected typhoid case was defined as diagnosis of IP or symptoms of fever, abdominal pain, and one or more of the following: vomiting, diarrhea, constipation, joint pain, headache, general body weakness, clinical suspicion of IP, or failure to respond to antimalarials in a Kasese resident from July 16, 2009-December 31, 2011 or in a Bundibugyo resident in 2011. Among Kasese residents, 658 suspected cases were identified; 519 were diagnosed with IP. Among Bundibugyo residents, 330 suspected cases were identified and 56 were diagnosed with IP. Laboratory surveillance from October -December 2011 isolated *Salmonella* Typhi by blood or stool culture from 9 Kasese and 15 Bundibugyo patients. Among 19 isolates tested for antimicrobial sensitivity, 1 had intermediate susceptibility to ciprofloxacin, 15 were multidrug resistant but sensitive to ciprofloxacin, and 3 were pan-susceptible to all antimicrobials tested. Several pulsed field gel electrophoresis patterns were shared by isolates from both districts, suggesting that the outbreak spread from Kasese to Bundibugyo. Untreated drinking water was suspected as the chief transmission route. Drinking water sources in areas of high typhoid incidence in both districts yielded *Escherichia coli*, an indicator of fecal contamination. Recommended control measures included emergency point-of-use water treatment interventions and community education about sanitation and hygiene.

1410

IDENTIFICATION OF ANTI-*SALMONELLA ENTERICA SEROVAR TYPHI* IMMUNE RESPONSES IN CHRONIC CARRIERS OF S. *TYPHI* IN KATHMANDU, NEPAL

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Salmonella enterica serotype Typhi can colonize and persist in the gallbladder of infected individuals. This can result in an asymptomatic chronic carrier state and chronic carriers can act as persistent reservoir of infection within a community. Unfortunately, little is known about host-pathogen interactions in the biliary tract of chronic carriers, and there is currently no reliable diagnostic assay to identify asymptomatic S. Typhi carriage. To address this, we applied an immunoscreening technique, *in vivo*-induced antigen technology (IVIAT), to identify potential biomarkers unique to S. Typhi chronic carriers. IVIAT identifies humorally immunogenic antigens expressed uniquely *in vivo*, and we hypothesized that S. Typhi surviving in the biliary tract of humans may express a unique proteomic profile. In brief, we generated a 120,000 clone genomic inducible expression library of S. Typhi CT18 (500-1500 bp fragments) in *E. coli* BL21DE3 and screened the library against pooled sera of patients (pre-adsorbed with *in vitro* grown S. Typhi and *E. coli* BL21DE3) who had bile cultures positive for S. Typhi at the time of elective cholecystectomy in Kathmandu. We identified 268 genes of interest from our primary screen, and subsequently sub-cloned each identified gene. Thus far, we have identified 50 proteins that are immunoreactive in S. Typhi carriers; these include a number of putative membrane proteins, lipoproteins, and hemolysin-related proteins. We are comparing these responses to those in patients with acute S. Typhi infection (typhoid fever) and patients from S. Typhi endemic zones with bile cultures negative for S. Typhi to identify uniquely immunoreactive antigens in Typhi carriers.

1411

IMPACT ASSESSMENT OF A MASS TYPHOID FEVER VACCINATION CAMPAIGN - FIJI, 2011

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Typhoid fever, a life-threatening disease, is endemic in Fiji. During June-December 2010, a mass typhoid vaccination campaign was conducted in Fiji targeting 65,000 persons ≥2 years old in cyclone and outbreak-affected areas. Considering limited use of typhoid vaccine in post-disaster or outbreak settings, we evaluated the campaign impact. We calculated confirmed typhoid incidence rates for 2008-11 using Fiji's national laboratory surveillance data. For all reporting subdivisions, we calculated risk ratios (RR) and 95% confidence intervals (CIs) for incidence in years post- (2011) versus pre-campaign (2008-9 annual average). The percentage of the population vaccinated was determined from campaign doses administered and medical area census populations; subdivision populations >20% vaccinated were called "vaccinated." In subdivisions with high pre-campaign incidence (≥100/100,000/year), we used log-binomial regression to estimate RRs and 95% CIs for the proportion of positive blood cultures in the high season months (January-August) post-(2011) versus pre-campaign (2008-10). Nationwide, 7% of the population was vaccinated, and confirmed typhoid was unchanged at 44/100,000/year between 2008-9 and 2011. In 11 unvaccinated subdivisions, post-campaign incidence was either unchanged, or significantly increased in 6 subdivisions (individual RRs ranged 2.2-7.8). In the 3 vaccinated subdivisions, post-campaign incidence was significantly decreased (individual RRs ranged 0.2-0.6). In the 2 high-incidence, unvaccinated subdivisions, the post-campaign proportion of positive-cultures increased (RR=1.8, CI=1.2-2.7; RR=1.6, CI=1.1-2.2). In three high-incidence, vaccinated subdivisions, the post-campaign proportion of positive-cultures decreased (RR=0.3, CI=0.1-0.6; RR=0.5, CI=0.3-0.9) or was unchanged (RR=1.4, CI=0.9-2.0). Post-campaign, confirmed TF cases in Fiji decreased in vaccinated areas and increased in unvaccinated areas. Typhoid vaccination can be considered in other high-incidence areas in Fiji and similar settings along with comprehensive typhoid control measures.

1412

ORIENTIA TSUTSUGAMUSHI, RICKETTSIA AND *LEPTOSPIRA* SPECIES AS CAUSES OF MENINGOENCEPHALITIS IN LAOS

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Rickettsial and leptospiral diseases have been recorded as rare causes of meningoencephalitis. We see such patients in the Lao PDR (Laos) where *Leptospira* spp., *Orientia tsutsugamushi* (scrub typhus), *R. typhi* (murine typhus), *Rickettsia* spp. (Spotted Fever Group) are important causes of fevers. There have been no prospective studies to determine the clinical importance and the epidemiology of rickettsial and leptospiral CNS infections in endemic countries using modern techniques. We therefore investigated the incidence of Leptospira, *Rickettsia* spp and *O. tsutusgamushi* among patients presenting with CNS infections to Mahosot Hospital in Vientiane, between 2003 and 2011. We performed paired MAT serology for anti-IgM/G Leptospira, paired IFA anti-IgM serology for rickettsial pathogens and cereberospinal fluid (CSF) and blood PCR for *Leptospira* spp., *Rickettsia* spp. and *O. tsutusgamushi*, by qPCR using 47kDa, 17kDa and rrs targets, respectively. We found evidence, using CSF PCR assays, for *O. tsutsugamushi*, *Leptospira* and *Rickettsia* spp. in 17/1030 (1.7%), 16/994 (1.6%) and 14/ 975 (1.4%) consecutive patients, respectively. In comparison to these 47 positive patients, CSF PCR for *S. pneumonia, N. meningitis* and *H. influenzae* b identified 38 patients in the same series with 'conventional' meningitis pathogens in CSF. These data suggest that scrub typhus, leptospirosis and murine typhus are important causes of CNS disease in Laos. The data underline the need for timely testing of patients with meningoencepahlitis for these 'atypical' pathogens. Such tests would be clinically important as rickettsial CNS disease would not be expected to respond to third generation cephalosporins that are commonly used for the empirical therapy of meningitis.

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LEPTOSPIROSIS IN MAMMALIAN RESERVOIRS AND SURFACE WATER IN ALTO MAYO VALLEY, SAN MARTIN, PERU

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Leptospirosis is caused by bacterial spirochetes of the genus *Leptospira.* All mammals can chronically shed *Leptospira* in their urine and humans

can become infected following contact with contaminated water or soil. In 2010, the Institute of Tropical Medicine of the Universidad Nacional Mayor de San Marcos found that 64.75% (CI 95%: 58.76-70.74) of rice field workers (n=261) in Alto Mayo Valley, Peru were seropositive for pathogenic leptospirosis by microagglutination test (MAT). The present study aimed to identify mammalian reservoirs and water sources of pathogenic leptospirosis in this region. In October 2011, at the start of the rainy season, serum and urine samples were collected from 179 domestic animals, including 57 dogs, 56 cows, 49 pigs, and 17 sheep from three rural settlements in the Alto Mayo Valley. In addition, 217 rodents, primarily *Mus musculas,* were trapped from rice fields and houses, and serum and kidneys were collected. Water samples were collected from 146 locations including rice fields (n=28), canals (n=47), standing water sources (n=45), and tap water (n=26). Epidemiological surveys were conducted (n=114) to identify risk factors associated with leptospirosis-positive households. MAT analysis of domestic animal and rodent sera is currently underway, as well as PCR of urine samples. To date, 14.29% (31/217) of kidney samples and one water sample (1/146) were positive by PCR for *Leptospira spp.* Genetic sequencing revealed that 2 of 31 rodent kidney samples were colonized by pathogenic *Leptospira interrogans*, while the remaining were colonized by the non-pathogenic species *Leptospira biflexia.* Water sources did not appear to be a significant source of leptospirosis prior to the rainy season. Preliminary results indicate that *Mus musculas* in rice fields may be a significant reservoir for leptospirosis in this region. Upon completion of all sample processing, this data will complement our understanding of the site-specific epidemiology of this disease and will provide information necessary for public health interventions in the Alto Mayo Valley.

1414

IMPACT OF A RURAL BANGLADESH SCHOOL WATER SANITATION AND HYGIENE INTERVENTION WITH AND WITHOUT ADDING HARDWARE

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To improve hygienic practices in Bangladesh, a countrywide school-based behavior change communication (BCC) intervention was implemented for 18 months that included hygiene promotion sessions taught by trained teachers; formation of student brigades engaged in maintaining clean school compound; and quarterly hand hygiene demonstration through street shows, fairs and rallies. In a subset of these schools the intervention added to or improved existing water, sanitation and hygiene (WASH) facilities along with the BCC. We evaluated whether BCC alone was sufficient, or if provision of WASH facilities combined with BCC was necessary to improve hygiene practices. We selected 800 intervention schools, 200 of which received combined interventions, and 600 control schools, each from 50 similar clusters, where the probability of selection was proportional to the size of the cluster. We interviewed 1400 headmasters and 5600 students. We calculated the risk difference (RD) adjusted for clustering for facilities and practices between combined and control schools and also between schools receiving only BCC and controls. We calculated difference in difference to estimate the effect of WASH facilities in addition to BCC. Fifty-six percent of combined intervention schools had clean water points with proper drainage compared with 42% of BCC only schools (p=0.004) and 35% of control schools (RD= 20; 95% CI= 11, 29). Of combined intervention schools, 64% had soap available inside/ near the toilet compared with 62% of BCC only schools (p=0.62) and 49% of control schools (RD= 16; 95% CI= 7, 25). Of combined intervention schools, 66% had clean toilets compared with 65% of BCC only schools (p=0.80) and 56% of control schools (RD=10; 95% CI=0.3, 20). When we asked students to demonstrate how they usually washed their hands, 52% of students from combined intervention schools washed both hands with soap compared with 54% of students from BCC only schools (p=0.32) and 42% of students from control schools (RD= 10; 95% CI=8, 18). Levels of hygiene practice and WASH facilities among all intervention schools were significantly better than the schools that did not receive any intervention. Behavior was no better in schools that received combined interventions compared with those that received only behavioral communication interventions. Behavioral communication messages may be a particularly cost effective approach to improving hand washing in schools.

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THE IMPACT OF IMPROVED SCHOOL WATER, SANITATION AND HYGIENE ACCESS ON PUPIL DIARRHEA: A CLUSTER-RANDOMIZED TRIAL

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Numerous studies have assessed the impact of improved access to water, sanitation, and hygiene (WASH) at the household level in reducing diarrheal disease, but few have rigorously assessed the impact of WASH in the school setting. Lack of access to improved WASH facilities and behaviors at school may increased risk of diseases due to the vulnerable age of children, increased opportunity for transmission of infectious agents, and lack of an immune response to organisms circulating in the public domain. We conducted a cluster-randomized trial to assess the impact of a school-based WASH intervention on diarrheal disease among primary school pupils. The study was carried out among 4,665 pupils in 185 public primary schools in Nyanza Province, Kenya. Two study populations were used: schools with a dry season water source within 1KM and those without. Schools with water nearby were randomly assigned to receive hygiene promotion and water treatment (HP&WT), HP&WT + sanitation, or no intervention (control). Schools without a nearby water source were randomly assigned to receive HP&WT, sanitation, and water supply improvements or no intervention (control). Our primary outcome was pupil-reported seven-day recall of diarrheal symptoms. At endline, pupils in schools with nearby dry-season water sources that received improvements in HP&WT and sanitation demonstrated similar measures of diarrhea period prevalence (RR 0.88, 95% CI 0.60-1.28) and diarrhea illness duration (IRR 0.85, 95% CI 0.57-1.24) compared to pupils attending associated control schools. Similar results were noted for pupils attending schools with HP&WT interventions only. Pupils attending schools without a water source in the dry season that received a water supply improvement followed by HP&WT and sanitation showed a 66% reduction in diarrheal disease (RR 0.34, 95% CI 0.17-0.64) and 70% reduction in days of illness (IRR 0.30, 95% CI 0.15-0.60) compared to associated controls. In settings with no water supplies in the dry season, an integrated school-based intervention to improve water supply, water quality, sanitation, and handwashing can reduce diarrheal illness among pupils. Since many schools in low-income settings function without year-round water supplies, these should be a priority for implementing WASH interventions.

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EVALUATION OF EDUCATION THROUGH LISTENING, A COMMUNITY ENGAGEMENT METHODOLOGY, TO PROMOTE THE ADOPTION OF SAFE HOUSEHOLD WATER TREATMENT BEHAVIORS IN COMMUNITIES IN WESTERN KENYA

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Household water treatment has been shown to reduce diarrhea risk by nearly 40%, but relatively low rates of adoption of these interventions have limited the scale at which they are being used. New behavior change approaches are needed to accelerate adoption. In 2010, we evaluated the impact of Education through Listening (ETL), a behavior change methodology, on the adoption of household chlorination. ETL is a community engagement technique that is a person-centered way of communicating and giving feedback to promote behavior change. We randomized 12 villages in Vihiga District, Kenya into an intervention group in which ETL was used to motivate home water chlorination and a comparison group that used the standard village-based social marketing approach promoted by the Safe Water and Aids Project, a local Kenyan non-governmental organization. Over a 6-month period, during biweekly home visits mothers were interviewed about reported water treatment and diarrheal disease in children <2yo; water treatment was confirmed by testing stored water for residual chlorine. A higher percentage of households in ETL villages than comparison households had reported (14% versus 11%, Pearson's chi-square, p = 0.03) and confirmed (7.5% versus 3.6%, Pearson's chi-square, p <0.0001) household water treatment with chlorine products. There was no difference in the proportion of children <2yo reported to have diarrheal disease between the intervention (6%) and comparison (6%) groups. However, the percentage of children with reported diarrheal disease was significantly lower in households that reported treating drinking water by any method than non-treating households (4% vs 7%, Pearson's chi-square, p=0.027). Although use of ETL appeared to increase the reported and observed use of chlorine products, adoption was modest. Further study of barriers to water treatment is needed.

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SOAPY WATER: A LOW-COST SOLUTION FOR HAND WASHING PROMOTION

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Cost, theft and difficulty in sharing are barriers to keeping soap at a hand washing station that hinder regular hand washing in low income communities. Soapy water, a solution of water and locally available detergent, contained in plastic soda bottles is a low cost alternative to bar soap. We piloted soapy water in rural communities and measured uptake. We enrolled rural Bangladeshi households with children age <3 years in 12 villages, for four study arms: promotion only (n=148), promotion plus handwashing stations with soapy water bottles (n=118), promotion plus handwashing stations with bottles plus detergent refills (n=107) and control (no products no promotion; n=73). Our hand washing stations (wholesale cost per unit USD 6.5) included a bucket fitted with a tap, a stool, a basin and a soapy water bottle. Health workers promoted hand washing, the convenience of having soap and water together and the utility of making and using soapy water in all study arms except the control. We collected data on handwashing resources and practices through observations and survey questions 3-4 months after commencement of the intervention. Soapy water or soap together with water was observed in 6% of (4/72) control households, 23% (26/116) of households with promotion only, 63% (65/103) of households with handwashing station plus bottles, and 75% (68/90) of households with station, bottles plus detergent. Intervention arms had significantly higher proportions of handwashing stations stocked with soap or soapy water compared to controls (p=<0.001 in all three arms). Additional intervention components were associated with significant increase in uptake: 40% (p<0.001) higher with stations plus bottles versus promotion only and 12% (p<0.04) higher with stations, bottles and detergent compared with stations plus bottles. Soapy water was an acceptable low cost hand washing agent alternative to bar soap in rural low income communities. Providing hand washing stations increased uptake of soapy water, but even in the absence of project provided detergent and hardware, households prepared this easily and kept it at the handwashing station. Soapy water may increase habitual handwashing by addressing key barriers such as cost, sharing and availability near water sources. This uptake should be further evaluated to assess its longer term impact on habits and health.

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MICROBIOLOGICAL EVALUATION OF THE EFFICACY OF SOAPY WATER TO CLEAN HANDS

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The high cost of bar soap relative to household income is an important barrier to handwashing in low-income communities. Soapy water made from powdered detergent is a low-cost alternative that could overcome these barriers. Among low-income households in Dhaka, Bangladesh, we compared the efficacy of handwashing with soapy water to washing with bar soap or water alone for removal of fecal indicator organisms from hands. We enrolled 84 mothers with at least one child < 5 and randomly assigned 28 mothers to each of three handwashing agents: water alone, bar soap and soapy water (30g of powdered detergent mixed with 1.5 liters of water). For each mother, field workers randomly selected the right or left hand to collect a hand rinse sample before handwashing and then collected a hand rinse sample from the opposite hand after washing. An unwashed hand rinse sample and a washed hand rinse sample were collected in each of 5 different visits: two after 15 seconds of washing with soapy water, two after washing with bar soap at two rubbing times (15s and 30s), and one after 15s rinsing with water alone. We assessed the concentration of thermotolerant coliforms in hand rinse samples (log CFU per hand) by membrane filtration, and used paired t-tests to compare these concentrations before and after handwashing with each agent. We collected 168 hand rinses each for soapy water and bar soap, and 84 hand rinses for water alone. Soapy water and bar soap removed thermotolerant coliforms effectively after 15s of rubbing (log mean reduction=0.66, p<0.001 for soapy water; and 0.58, p=0.001 for bar soap). Increasing rubbing time from 15s to 30s did not significantly alter the microbiological efficacy of soapy water or bar soap (log mean reduction of 15s minus log mean reduction of 30s =0.04, p=0.48 for soapy water; and 0.08, p=0.53 for bar soap). Washing hands with water alone also reduced thermotolerant coliforms (log mean difference=0.30, p=0.029). Washing hands with soapy water was more effective than washing hands with water alone in reducing thermotolerant coliforms (difference in log mean reduction = 0.35, p=0.048).Soapy water is more effective than water alone and as effective as bar soap in removing indicator organisms from hands. Washing for 15s is sufficient to remove bacteria from hands with bar soap and soapy water. In low-income communities, washing hands with soapy water can be promoted as an effective, low-cost alternative to bar soap.

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CHRYSOMYA PUTORIA, A PUTATIVE VECTOR OF DIARRHEAL DISEASES

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Chrysomya spp are common blowflies in Africa, Asia and parts of South America and some species are generated in prodigious numbers from pit latrines. Because of their strong association with human faeces and their synanthropic nature, we examined whether these flies are likely to be vectors of diarrhoeal pathogens. Flies were sampled using exit traps placed over the drop hole of latrines in Gambian villages. A median of 12.5 flies/latrine/day (IQR=0.0-86.0) was collected, of which 95% were *Chrysomya* spp, nearly all *C. putoria*. Odour-baited traps were used to determine the relative attractiveness of different breeding media and foods to these flies. More flies were collected from traps with faeces from young children (median=2.5, IQR=1.0-8.5) and dogs (median=1.0, IQR=0.0-12.0) than from herbivores (median=0.0, IQR=0.0-0.0; calf, cow, goat and horse; p<0.001). Flies were strongly attracted to raw meat (median=44.5, IQR=26.2-143.0) and fish (median=0.0, IQR=0.0-19.8) compared with cooked and uncooked rice, and mangoes (median=0.0, IQR=0.0-0.0; p<0.001). The presence of bacteria in wild caught flies was confirmed by culture and bacterial DNA was identified using PCR. *Escherichia coli* were cultured from the surface of 21% of *Chrysomya* and 10% were enterotoxigenic (ETEC). Enteroaggregative *E. coli* (EAEC) were identified by PCR in 2% of homogenized *Chrysomya* spp, *Shigella* spp in 1.4% and *Salmonella* spp in 0.6% of samples. The large numbers of *Chrysomya* that can be produced from pit latrines, the presence of enteric pathogens on flies, and their strong attraction to raw meat and fish suggests these flies may be important vectors of diarrhoeal diseases in Africa.

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QUANTITATIVE PCR-BASED DETECTION OF PATHOGENIC *LEPTOSPIRA* IN SLUM WATER

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Leptospirosis has emerged as a major public health problem in urban slum settlements worldwide. Environmental surface water is an important reservoir for disease transmission in this setting. Pathogenic *Leptospira* have been detected in surface water from slum communities. However the ecological factors which influence the spatial and temporal dynamics of leptospires in this reservoir remain poorly understood. We performed a one-year longitudinal survey of leptospires in environmental surface water in an urban slum community, which was situated in a valley of 0.1 km^2 in the city of Salvador, Brazil. Pooled water and sewage samples were systematically collected from study households during a two-week period in the months of July, October and January. A lipL32-based qPCR assay was used to determine genome equivalents of leptospires in DNA extracted from 50ml samples. We detected leptospires in 12% (61) from 498 surface water samples collected during two survey periods of July and October. The proportion of qPCR positive samples (18% vs. 9%, P<0.05) and leptospiral concentration (10.1 vs. 6.6/ml, P<0.05) were significantly increased for the month where rainfall was greater (October vs. July; 230 vs. 81mm). Samples collected in the morning were significantly more frequently positive (17% vs. 6%) and had higher leptospiral concentration (9.3 vs. 5.8/ml) than those collected in the afternoon samples. The proportion of qPCR positive samples and leptospiral concentrations were also significantly higher in sewage (18%; 8.8/ml) than pooled water (6%; 6.2/ml). These findings indicate that the diurnal and seasonal variations influence the dynamics of leptospires in the environment. Furthermore they also suggest sewage may be a key transmission source in slum communities, and interventions targeting this reservoir will be necessary for effective prevention.

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APPLICATION OF NANOTRING™ TECHNOLOGY TO MEASURE CHANGES IN GENE EXPRESSION IN *PLASMODIUM FALCIPARUM*

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Experiments that investigate differential gene expression have traditionally taken a gene-by gene approach (using quantitative real-time PCR) or a genome-scale approach (using microarrays). Nanotring™ technology is based on direct multiplex measurement of gene expression, effectively "counting" transcripts using barcoded probes and single molecule imaging. This approach offers a middle-level throughput to assay hundreds of transcripts simultaneously, using much less material than a microarray. We sought to apply Nanostring™ to measure gene expression in *Plasmodium falciparum* culture-adapted parasites and patient samples. We designed a custom codeset of 328 genes that distinguish between stages of the malaria asexual life cycle as well as between distinct transcriptional profiles previously observed in parasites isolated directly from infected patients. Using this subset of genes we were able to distinguish between asexual life cycle stages using small volumes (10uL parasitized red blood cells) of cell lysate. Life cycle stage correlations between Nanostring™ and microarray data were maintained with as few as 10,000 parasites. Direct patient samples, containing an abundance of human RNA, showed good correlation with microarray data gathered from the same samples. Even at parasitemia levels relevant to human infection, life cycle correlations were very strong. Whole genome imputation from the codeset for direct patient samples was also performed. Overall, Nanostring™ performs well with very small amounts of both cell lysate and extracted RNA, and constitutes a highly sensitive, enzyme-free approach to measuring gene expression in the malaria parasite. This tool could be ideal for screening patient samples prior to performing in-depth RNA sequencing, or as independent data for studies of parasite physiology during drug treatment or other experiments.

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COPY NUMBER VARIATION WITHIN A NATURAL POPULATION OF *PLASMODIUM FALCIPARUM*

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Copy number variation is a key evolutionary mechanism for gene evolution and diversification. In *Plasmodium falciparum*, it is known to play important roles in virulence and drug resistance. Copy number variants (CNVs) have been extensively studied in culture-adapted laboratory strains. However, the genome-wide extent of CNVs in natural populations is not well understood. In order to address this we have analyzed over 30 short-term cultured field isolates from Senegal. Using whole-genome next-generation sequencing, using our novel correction algorithms for

sequencing biases and a mean shift approach to delineate CNVs allows the precise demarkation of CNVs, often to the base pair resolution. We find that on average the number of CNVs > 1kb in a strain was 158 of which 38 were duplications and 122 deletions. The vast majority of CNVs fall within the virulence genome compartment (e.g. var, rifin and stevor gene families and subtelomeric regions) highlighting their important role in host evasion. The higher proportion of deletion CNVs is mainly due to inadequate remapping of highly polymorphic var genes and as such do not strictly represent a deletion at the given var nor a reduced var complement. The core genome is relatively invariant compared to the virulence regions. It also appears less variant relative to culture-adapted strains suggesting variation may be selected for or more tolerated in such settings. Many of the core CNVs detected are shared within the Senegalese population, indicating either regional selection or the 3D7 reference genome being the rare variant. Interestingly several isolates demonstrated extensive and markedly-elevated read depth within the subtelomeric var regions - over three times the CNV content - suggesting that the virulence compartment may vary more extensively then previously appreciated. Together with our ongoing experimental validation we will present a detailed picture of the pattern and nature of copy number variation within this important pathogen.

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PLASMODIUM COATNEYI CAUSES SEVERE ANEMIA AND INFLAMMATION IN BONE MARROW AND OTHER ORGANS OF RHESUS MACAQUES

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Plasmodium coatneyi infection in rhesus macaques has been considered a relevant animal model of *P. falciparum* infection in humans, due to its tertian periodicity, tissue sequestration features, and severe disease outcomes. In particular, *P. coatneyi* infection in rhesus macaques has been proposed for studies of cerebral malaria, with sequestration in the brains of infected rhesus macaques and associated clinical sequelae. However, the clinical features of this infection, including neurological syndromes, have not been recently evaluated for their similarity to severe human malaria disease. We conducted a pilot infection of six rhesus macaques with blood-stage *P. coatneyi* (frozen stabilate obtained at 7.05% parasitemia; strain deposited in MR4 by Dr. W. Collins) and obtained clinical, immunological, pathological, and parasitological data longitudinally. The infection was allowed to progress in individual monkeys until pre-determined clinical endpoints appeared, including any features of severe malaria. All monkeys developed severe anemia with a >60% drop from baseline hematocrit (final hemoglobin levels 3.2 - 5.6 g/dL) twelve to fourteen days post-infection and displayed peak parasitemias between 6.5 and 12.45%. Animals demonstrated lassitude and withdrawal at higher parasitemias, but not convulsions, unresponsiveness, or focal neurologic signs. Phagocytic cells and red blood cells containing pigment were observed in several organs without prominent parenchymal changes, including cerebral vessels without evidence of ischemia. T cell activation and proliferation, together with pigment-laden macrophages, were evident during in peripheral blood and tissues, including bone marrow. *P. coatneyi* infection in rhesus macaques routinely causes acute severe anemia, which may be useful for future mechanistic studies of this common severe malaria manifestation in humans.

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IMPAIRED SKELETAL MUSCLE MICROVASCULAR FUNCTION AND INCREASED SKELETAL MUSCLE OXYGEN CONSUMPTION IN SEVERE *FALCIPARUM* MALARIA

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Organ dysfunction in severe *falciparum* malaria (SM) is associated with tissue hypoxia, which results from an imbalance between oxygen supply and demand. In SM, microvascular obstruction from parasite sequestration results in impaired oxygen delivery. However, microvascular function (capacity to increase oxygen delivery in response to ischemia) and oxygen consumption have not been assessed in host tissue. We used near-infrared resonance spectroscopy (NIRS) to measure tissue oxygen saturation (StO_2), combined with an ischemic stress to compare microvascular function (StO_2recov) and oxygen consumption (VO_2) in thenar muscles among adults in Papua, Indonesia with SM (n=36), moderately-severe malaria (MSM; n=33), severe sepsis (n=24) and healthy controls (HC; n=36). Mean StO_2recov (skeletal muscle reoxygenation rates) was 16% and 22% lower in SM (2.7%/s) compared to MSM (3.1%/s) and HC (3.5%/s) ($p<0.001$), and comparable to severe sepsis (2.5%/s). In SM, StO_2recov inversely correlated with venous lactate ($r=-0.63$; $p<0.001$) after adjustment for disease severity. StO_2recov was a significant predictor of death (ROC: 0.71[95%CI: 0.51-0.92]), with each percentage decrease associated with an increased risk of death (OR 2.49 (95%CI 1.05-6.2). In contrast, VO_2 was increased in SM by 8% compared to MSM and 18% with HC and sepsis ($p<0.001$), and associated with parasite biomass (plasma HRP2); $r=0.49$, $p=0.04$. Microvascular function is impaired in SM and associated with increased mortality, while oxygen consumption is increased. Tissue hypoxia and organ dysfunction may arise not only from parasite sequestration and heterogeneous microvascular obstruction, but also from impaired functional ability of the microvasculature to match oxygen delivery to increased oxygen demand.

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USING LABORATORY AND SEASONAL DIFFERENCES IN RETINOPATHY NEGATIVE VERSUS POSITIVE CEREBRAL MALARIA TO IMPROVE UNDERSTANDING OF DISEASE PATHOPHYSIOLOGY

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Children with cerebral malaria (CM) can be categorized by the presence or absence of malaria retinopathy. We compared admission laboratory, demographic, and seasonal data between children admitted with retinopathy positive versus negative and used these comparisons to gain insight into the underlying pathophysiology of retinopathy negative CM. We retrospectively reviewed admission laboratory and clinical parameters and the seasonal pattern of disease presentation in patients admitted with CM in Blantyre, Malawi from 1997--2010 and compared these data across retinopathy status. Patients with retinopathy negative CM had higher glucose concentrations, hematocrits, platelet counts, and lower lactate concentrations and peripheral parasite counts than those with retinopathy positive CM. Children with retinopathy negative CM were more likely to be in deeper coma upon admission than those with malaria retinopathy. The seasonal pattern of disease presentation also varied by retinopathy

status. Taken together, these findings support the hypothesis that these conditions have different underlying etiologies. Acute *Plasmodium falciparum* infection is likely not sufficient to produce the retinopathy negative CM syndrome.

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MOLECULAR PATHOLOGICAL INVESTIGATIONS OF FATAL *PLASMODIUM FALCIPARUM* MALARIA

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To investigate the pathophysiology of fatal human malaria using molecular pathology techniques, we are conducting an autopsy study in Beira, Mozambique, examining malaria and control deaths in children and adults. Full clinical and autopsy based clinicopathological correlation determined the spectrum of clinical complications of severe malaria and cause of death. Tissues from different organs were used to extract total mRNA and microRNA. Whole genome and miRNA expression profiles were generated using the Illumina Human-12 V4 BeadChip array and Affymetrix GeneChip miRNA array v2 respectively. An initial screening analysed total mRNA and microRNA transcriptomes from the brain of patients dying of fatal malaria and non-malaria control deaths (both n=3, 3 separate brain regions, samples = 9 per group). Clustering analysis showed no significant differences between three brain regions. A total of 223 mRNAs and 54 miRNAs were significantly differentially expressed in malaria (using cutoffs of fold difference x1.5, and $p<0.05$). The network, functional and canonical pathway analyses were generated using Ingenuity software pathway analysis. Integration of the putative mRNA targets of differentially regulated miRNAs with mRNA expression data from the same specimens revealed a wide number of enriched functions and pathways, mainly associated with host immune responses, cellular morphological changes and cell death regulation. Gene families which were significantly upregulated included pathways of cell signalling and transmigration, inflammation and cellular homeostasis. Transcripts encoding markers of neuronal damage, such as S100 and APP, were highly increased, as were the chemokine MCP-1, cytokines Ang-4, IL-6 and IL-17 (but not TNF or IFN-γ). Hypoxic inducible molecules such as C7orf68 were increased, and mediators of cerebral oedema formation, such as aquaporin 4 and fibrinogen. There was downregulation of several genes stimulating cell death and neuronal apoptosis. Neurotransmitters and proteins involved in synaptic function and stabilization or microtubule formation were downregulated, such as PENK (proenkephalin precursor A). This study, the first integrated analysis of miRNA and mRNA expression profiling in fatal P.*falciparum* malaria, represents a proof of concept for using molecular techniquea on autopsy tissues to understand the pathology and pathogenesis of human malaria.

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SURROGATE MRI MEASURE (SAMKAM RATIO) PREDICTS OUTCOME IN PEDIATRIC CEREBRAL MALARIA

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Pediatric cerebral malaria (CM) increases both brain volume and intracranial pressure (ICP). The mortality from CM is 15-25% even with highly attentive care. Massive increases in brain volume, assessed by magnetic resonance imaging (MRI) and interpreted by radiologists, are strongly associated with fatal outcomes in Malawian children with cerebral malaria. We developed a surrogate measure of brain volume for use by non-radiologists, and evaluated its utility, using images generated by 0.35T GE Signa Ovation scanner . The SamKam ratio is calculated using the height of the right parietal lobe on the first coronal T2 slice behind the splenium divided by the peri-brainstem CSF. The latter is the sum of the measurements of the CSF anterior and posterior to the brainstem at the level of the 4th ventricle apex, measured on the midsagittal section (T1). Three independent observers calculated the ratio on the same 20 scans. Pearsons correlation coefficients were calculated and were greater than 0.86 for all combinations. When SamKam ratio is used to predict severe brain swelling as measured by two independent radiologists the AUROC is 0.75. During the 2009 and 2010 seasons, 120 Malawian children with retinopathy-positive CM underwent brain MRI scanning on admission and daily thereafter until death or discharge(Age 9-168mo, mean 48mo; 45.8% male). There were twenty fatalities, and in 85%, the admission SamKam ratio was > 6.5. SamKam ratios declined over time in survivors with serial scans (n=*8).* The SamKam ratio may be used to identify CM patients increased brain volumes when radiologists are not available.

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USE OF GEOSPATIAL MAPPING MODELS TO ACCURATELY PREDICT *SCHISTOSOMA MANSONI* PREVALENCE IN NYANZA PROVINCE, KENYA

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Schistosomiasis, a parasitic disease that affects over 200 million people, can lead to significant morbidity and mortality; distribution of single dose preventative chemotherapy significantly reduces disease burden. Implementation of control programs is dictated by disease prevalence rates. For *Schistosoma mansoni,* infection prevalence is determined by costly and labor intensive screening of stool samples. Because ecological and human factors are known to contribute to the focal distribution of schistosomiasis, we sought to determine if specific environmental and socioeconomic factors could be used to accurately predict *S. mansoni* prevalence. We designed a mixed model to assess associations with *S. mansoni* rates in schools and controlled for spatial autocorrelation. Data on *S. mansoni* prevalence, school name, and GPS location of the school were obtained from 457 primary schools in Nyanza province in western Kenya. *S. mansoni* rates were calculated through examination of stool samples from children in the selected school; the median number of children tested per school was 42 (range 9-80). Geographic layers for environmental and population features, such as water source proximity, poverty rates, land elevation, and soil type, were obtained from publicly available sources. Mapping models were constructed using ArcGIS 10 and R 2.13.0. Higher *S .mansoni* rates were associated with closer distance (km) to Lake Victoria (prevalence ratio = 0.75, 95% CI = 0.73-0.77), increasing soil pH (0.83, 0.79-0.86), and increasing monthly rainfall (mm; 0.991, 0.989-0.993). Distance to health facility, human influence index, poverty rate, and agricultural land use were not significantly associated with *S. mansoni* rate. Our mapping model suggests that easily assessable geographic data can be used by schistosomiasis control programs to accurately predict schistosomiasis prevalence. Development and use of these prevalence maps will allow control programs to plan and prioritize efficient control campaigns to decrease schistosomiasis burden.

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EVALUATION OF A NOVEL RAPID DIAGNOSTIC TEST FOR SCHISTOSOMIASIS HAEMATOBIUM (RDT-SH) BASED ON THE DETECTION OF HUMAN IMMUNOGLOBULINS BOUND TO FILTERED *SCHISTOSOMA HAEMATOBIUM* EGGS

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Schistosomiasis haematobium is a major cause of morbidity in Africa and the Middle East. A rapid diagnostic test for *Schistosoma haematobium* is needed to facilitate diagnosis and treatment, assist with disease surveillance and guide public health interventions. We evaluated a rapid diagnostic test for *S. haematobium* (RDT-Sh), a novel method for diagnosing *S. haematobium* infection. *S. haematobium* eggs are highly immunogenic and excreted into the urine coated in human IgG. We filtered 160 urine samples from children in the Kwale distract of Kenya to isolate eggs and used anti-human IgG antibody conjugated to horseradish peroxidase to bind to the human IgG attached to the eggs. We then added 3,3'5,5'-tetramethylbenzidine base (TMB) _ which turns blue in the presence of horseradish peroxidase _ to detect the presence of *S. haematobium* eggs. The RDT-Sh was compared in a double-blinded manner to the gold-standard method of diagnosing infection by urine microscopy. The RDT-Sh was positive in 89% of urine samples containing >1 egg/10mL (58/65 samples) and 97% of urine samples containing >11 eggs/10mL urine (35/36 samples) seen by microscopy. The RDT-Sh was negative 79% of the time when no eggs were seen on urine microscopy, but because up to three times more urine was used for the RDT-Sh, there were likely cases in which eggs were on the RDT-Sh filter but not detected by microscopy. We used latent class analysis incorporating urine microscopy, hematuria, proteinuria, and RDT-Sh results to determine an overall 97% sensitivity and 78% specificity for RDT-Sh, 96% and 81% for urine microscopy, 71% and 98% for microscopic hematuria, and 46% and 89% for proteinuria, respectively. The RDT-Sh is quick, inexpensive and easy to perform in the field for the diagnosis of *S. haematobium*. The RDT-Sh is able to detect all but the lightest of *S. haematobium* infections with a high degree of accuracy.

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URINE FOAM AS A MARKER FOR INFECTION WITH SCHISTOSOMIASIS HAEMATOBIUM

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The Baddorf-Sheele (BS) shake test measures urine foam to help diagnose Schistosomiasis haematobium in the field. The BS-shake test was performed by shaking 20mL of urine in a 50mL test tube as vigorously as possible by hand for approximately five seconds. Immediately after shaking, the height of the foam inside the test tube was recorded and these results compared to urine microscopy counts of *Schistosoma haematobium* eggs. The average height of the urine foam for study subjects with 17 to >1000 eggs/10mL urine was at the 36.4mL (SD 1.35) mark on the test tube, and for subjects with 0 eggs/10mL urine was at the 32.2mL (SD 2.36) mark. The sensitivity and specificity of the BS-shake test (positive when foam was measured at or above 34mL) was 74% and 72%, for microscopic hematuria 61% and 97%, and for proteinuria 43% and 83%, respectively, compared to microscopy. When >17 eggs/10mL urine were present, the BS-shake test, microscopic hematuria, and proteinuria were positive in 100%, 90%, and 80% of cases, respectively. Combining hematuria and the BS-shake test results detected 87% of samples with eggs seen on microscopy. The current gold standard test requires slide preparation, a trained technician, access to a microscope, and significant time and resource costs. A more easily performed and cost effective, though still reliable test is needed, especially for field studies and large public health screenings. The BS-shake test is an inexpensive, quick, and easy way to diagnose *S. haematobium* in endemic areas.

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TOWARDS THE DEVELOPMENT OF A RAPID DIAGNOSTIC TEST (RDT) FOR DETECTION OF ANTI-SCHISTOSOME ANTIBODIES

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Mass drug administration (MDA) of praziquantel is widely used in control programmes for both *Schistosoma mansoni* and *S. haematobium* infections. Different MDA strategies are used depending on how prevalent infection is in a given area. The Kato-Katz method is used for mapping *S. mansoni* infections, and questionnaires, detection of macrohaematuria and/or urine filtration methods for *S. haematobium*. Parasitological methods of diagnosis are however relatively insensitive, often misdiagnosing the infected as uninfected, and so prevalence is often underestimated. This can lead to the implementation of an inappropriate treatment strategy for a given community. The problem of underestimating prevalence is likely to become exacerbated in areas where praziquantel treatment has taken place, since the number of lighter infections which parasitology cannot detect is likely to increase. The need for more sensitive diagnostic assays is therefore greater than ever and it is envisaged that antibody-detection methods are likely to become increasingly useful. Indeed, they are already widely used in travellers' medicine clinics and have been integrated into the Chinese national control programme for *S. japonicum*. To be useful in schistosome-endemic areas however, a diagnostic test needs to meet the ASSURED criteria (particularly with regard to Affordability and User-friendliness), and so we are developing a rapid diagnostic test (RDT) that works by detection of anti-schistosome antibodies in human blood. Preliminary results indicate that this RDT could be useful for diagnosis of both *S. mansoni* and *S. haematobium* infections, and its low cost could make it useful not only for mapping purposes but also for diagnosis at the individual patient level.

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EVALUATION OF POINT-OF-CONTACT CIRCULATING CATHODIC ANTIGEN ASSAYS FOR THE DETECTION OF *SCHISTOSOMA MANSONI* INFECTION IN LOW AND MODERATE PREVALENCE SCHOOLS IN WESTERN KENYA

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Increased attention to schistosomiasis control efforts has highlighted the need for improved field diagnostics where rapid screening and mapping of *Schistosoma mansoni* infection guide control efforts. A urine point-of-contact circulating cathodic antigen (POC/CCA) assay manufactured by Rapid Medical Diagnostics (Pretoria, South Africa) has shown promise in areas where prevalence of schistosomiasis is high, but the assay has not been evaluated extensively in areas where prevalence is low. To evaluate the performance of the POC/CCA assay in areas of low to moderate prevalence, we tested primary school children for schistosomiasis in the Asembo region of western Kenya, using two versions of the POC/CCA assay_one commercially available and one experimental formulation_as well as duplicate Kato-Katz stool examinations and an anti-schistosome IgG ELISA. Latent class analysis was used to estimate sensitivities and specificities of the individual tests at each of three school prevalence levels: <10%, 10-25%, and >25%. Respective sensitivities and specificities of the POC/CCA assays among all participants (n=1798) were 93.9% and 57.8% for the commercial test and 76.9% and 89.8% for the experimental test.

The commercial POC/CCA assay was found to be most sensitive overall, but the experimental POC/CCA assay offered the best combination of sensitivity and specificity (82.0% and 91.4%, respectively) in the lowest prevalence zone. Both POC/CCA assays demonstrated positive correlation with infection intensity (as measured by egg count). The commercial POC/CCA assay was also evaluated for consistency and for measurement of treatment outcome, demonstrating substantial agreement across three daily administrations and reductions in POC/CCA band intensity one week after treatment. As intervention programs move toward sustained control and elimination, a diagnostic assay's abilities to perform in areas of low prevalence becomes paramount. Our findings suggest that the experimental POC/CCA assay may be a field-friendly alternative to the Kato-Katz exam in low prevalence settings.

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COMPARING HIGH-THROUGHPUT QUANTITATIVE DETECTION OF *SCHISTOSOMA*-DNA USING REAL-TIME PCR AND EXTENSIVE MICROSCOPY IN URINE SAMPLES FROM PRIMARY SCHOOL GIRLS IN COASTAL KWAZULU NATAL

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Of 300 million women and girls in Africa at risk of schistosomiasis, those most vulnerable to infection are pre-school and primary school children, adolescent girls and women of childbearing age. Genital schistosomiasis is reported as a complication among children. Since the diagnosis of female genital schistosomiasis (FGS) is difficult among children, diagnosing urinary schistosomiasis may help identify endemic areas for mass treatment where young girls are at risk for FGS. The diagnosis of schistosomiasis from urine may be challenging since haematuria and egg excretion are variable particularly in adults and in children with light infections with low levels of egg excretion. The aim was to compare two diagnostic tests using urine samples: real-time PCR for detection of *Schistosoma*-genus DNA and extensive microscopy as a practical tool for multiple exploration. Urine samples were collected on three consecutive days from 688 girls, aged 10-12 years, during a cross-sectional study in 18 primary schools. Quantification of *Schistosoma*-specific DNA was performed on a 200 µL aliquot of the first day urine, using a custom-made automated handling system for high through-put DNA isolation and PCR set-up. Full microscopy (3 days 2x10mL urines) was done on 621 (90.3%) and day 1 microscopy (2x10 mL urines) was done on 677 (98.4%) of the participants. Only 250 (36.3%) showed eggs in all 10 mL examinations collected over 3 days, while 210 (30.5%) were positive in only one out of six screenings collected on day 1. In addition, the number of eggs counted varied highly from day-to-day. *Schistosoma* DNA was detected using 200 µl of urine in 197 (28.6%) urine samples and DNA loads corresponded significantly with the average intensity of infection determined by microscopy. Also at school level, PCR determined *Schistosoma* infection reflected the focal distribution of disease transmission seen after extensive microscopy. The automated system facilitated high throughput quantification of *Schistosoma*-specific DNA loads in urine. In addition only 200 µl urine samples were required to achieve a sensitivity similar to extensive and labour intensive microscopy on consecutively collected large volume samples. The described PCR set-up could be used as a relatively straightforward laboratory-based procedure to assess the distribution of schistosomiasis in one urine only for large study populations, identifying communities at risk.

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COMPARISON OF DIAGNOSTIC METHODS AGAINST PCR FOR THE DETECTION OF *SCHISTOSOMA MANSONI* AMONG SCHOOL CHILDREN IN WESTERN KENYA

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The most widely used tools for detection of *Schistosoma mansoni* infection, stool examination and serology, are limited by low sensitivity or inability to distinguish between current from former infections, respectively. As a result, there is not an accepted "gold standard" for *S. mansoni* diagnosis for evaluation of new diagnostic assays. However, recent development of a semi-quantitative PCR that detects schistosome DNA in stool provides a tool for this purpose. We utilized the PCR method to evaluate a point of contact (POC) test designed to detect circulating cathodic antigen (CCA) in urine of persons infected with *S. mansoni*. School children (n = 1898) aged between 8-12 years from villages in western Kenya provided 3 stool and 3 urine samples on consecutive days for testing by Kato-Katz and a commercially available POC-CCA cassette. A portion of the first day's stool was preserved in ethanol and subsequently tested for presence of schistosome DNA by PCR(n=950). In addition, serum from a single blood sample was tested by ELISA for adult worm antigen-specific IgG. Children who were infected with *S. mansoni* were treated using praziquantel. A single urine sample was collected 1 week later for post-treatment POC-CCA testing. Compared to PCR, the single day POC-CCA urine test had an average sensitivity of 81.7% and an average specificity of 54.4%. Average single day Kato-Katz sensitivity was 58.2% and average specificity was 91.5%. When testing from the 3 days was combined, the POC-CCA sensitivity was 92.1%, and the Kato-Katz sensitivity was 70.4%; 3 day specificities of these tests were 68.7% and 92.1%, respectively. The ELISA was 52.6% sensitive and 85.1% specific. There were 675 children that were initially positive by POC-CCA and provided a urine sample 1 week after treatment with praziquantel; 461 (68.3%) of these children demonstrated decreased POC-CCA band intensity following treatment. Comparison of *S. mansoni* diagnostic tools demonstrates attributes and limitations of each test. Further development to optimize detection methods for *S. mansoni* is needed.

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MALARIA PREVENTION IN PREGNANCY IS ASSOCIATED WITH REDUCTIONS IN LOW BIRTH WEIGHT AND NEONATAL MORTALITY: A META-ANALYSIS OF 32 NATIONAL CROSS-SECTIONAL DATASETS IN AFRICA

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Low birth weight (LBW) is a significant risk factor for neonatal death. A prominent cause of LBW is *Plasmodium falciparum* infection during pregnancy. Intermittent preventive therapy (IPTp) and insecticide-treated mosquito nets (ITNs) have been shown by randomized trials to significantly reduce the risk of LBW in areas of stable transmission. We created a retrospective birth cohort from 32 national cross-sectional datasets in 25 African countries from 2000-2010 to examine the association of malaria prevention in pregnancy (IPTp and/or ITNs) with LBW and neonatal mortality under routine program conditions. An important innovation in this meta-analysis is the substantial effort made to limit potential selection

bias through exact matching on confounding factors associated with both exposure to malaria prevention in pregnancy and birth outcomes. A logistic regression model was used for assessing the association of malaria prevention in pregnancy on LBW, while a Poisson model was used for the outcome of neonatal mortality. Both models incorporated the matched strata as a random effect, while accounting for additional confounding factors with fixed effect covariates. Exposure of women in their first or second pregnancy to malaria prevention with IPTp and/or ITNs was significantly associated with decreased risk of neonatal mortality [Incident rate ratio = 0.820; 95% Confidence interval (CI): 0.698-0.962], compared to women with no protection. Compared to no protection, exposure of pregnant women during their first 2 pregnancies to malaria prevention in pregnancy through IPTp and/or ITNs was significantly associated with reduced odds of LBW, as measured by a combination of weight and perceived birth size [adjusted odds ratio = 0.792; 95% CI: 0.732-0.857). These data show malaria prevention in pregnancy to be associated with substantial reductions in neonatal mortality and LBW under routine malaria control program conditions, and for the most part are consistent with the efficacy results from controlled trials.

1436

A TRIAL OF INTERMITTENT SCREENING AND TREATMENT AS AN ALTERNATIVE TO INTERMITTENT PREVENTIVE TREATMENT WITH SULFADOXINE-PYRIMETHAMINE FOR THE CONTROL OF MALARIA IN PREGNANCY

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The incidence of malaria, including the incidence in pregnant women, is declining in some African countries, and resistance to sulfadoxine-pyrimethamine (SP) is widespread. Thus, intermittent preventive treatment in pregnancy with SP (SP-IPTp) may no longer be appropriate in certain situations, and alternative strategies are needed. A randomised, multi-centre controlled trial has been undertaken in four west African countries, including 5000 pregnant women who slept under an insecticide treated bed net. The standard SP-IPTp regimen (two to three courses of SP in the second and third trimester) will be compared to intermittent screening and treatment (IST) of parasitaemia using a rapid diagnostic test at scheduled antenatal clinic visits in the second and third trimester. The primary end points of the trial are prevalence of low birth weight (LBW), mean maternal haemoglobin at 38 ±2 weeks of gestation and prevalence of placental malaria. Other outcomes affecting mothers (anaemia, parasitaemia, clinical malaria) and children (still births, perinatal mortality) will also be analysed.The study was powered to show non-inferiority of IST compared to SP-IPTp with respect to prevalence of LBW. Recruitment of study participants is complete. Analyses will be finalised in the third quarter of 2012 and available to present at ASTMH in November. The study will provide information to national malaria control programmes in countries whether there are alternative, safe and effective methods to the WHO recommended SP-IPTp regimen for managing malaria in pregnancy. This could have particular important implications for the control of malaria in pregnancy in areas with high levels of SP resistance.

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EFFECTIVENESS OF INTERMITTENT PREVENTIVE TREATMENT WITH SULFADOXINE-PYRIMETHAMINE IN PREGNANT WOMEN IN WESTERN KENYA: RESULTS OF AN OBSERVATIONAL STUDY

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Intermittent preventive treatment with sulfadoxine pyrimethamine (IPTp) remains a key strategy for malaria prevention in pregnant women living in malaria endemic regions. However, increasing SP resistance threatens IPTp effectiveness. We assessed IPTp effectiveness in an area of western Kenya where *Plasmodium falciparum* malaria transmission is intense and resistance to SP is high. From August 2008 to June 2009, women delivering at two district hospitals were enrolled in a cross-sectional survey. We collected information on obstetric history, IPTp use (self-report or as recorded in the antenatal card), insecticide treated net use, and antimalarial treatment during pregnancy. At delivery, we measured the prevalence of maternal anemia (Hb< 8g/dL), peripheral parasitemia, placental parasitemia (impression smear) and low birth weight (LBW) (multivariate analysis pending). Overall, 977 HIV-negative women were enrolled and included in this analysis. Of these, 637 were gravida 1 or 2 and 340 were gravida 3+. Among women who were gravida 1 or 2, anemia prevalence by number of IPTp doses received was 14%, 11%, 7% and 2% for 0, 1, 2, and 3+ IPTp doses respectively (p<0.01); peripheral parasitemia prevalence was 19%, 12%, 12% and 7% for 0, 1, 2, and 3+ IPTp doses received respectively (p=0.07); placental parasitemia prevalence was 22%, 12%, 13% and 8% for 0, 1, 2, and 3+ IPTp doses received respectively (p=0.04); and LBW prevalence was 5%, 11%, 9% and 9% for 0, 1, 2, and 3+ IPTp doses received respectively (p=0.73). Among multigravidae, we found no significant reduction in the prevalence of anemia, or peripheral or placental parasitemia with increased number of IPTp doses; LBW prevalence was 11%, 6%, 3% and 0% for 0, 1, 2, and 3+ IPTp doses received respectively (p=0.02). Among gravida 1 or 2, IPTp was associated with a reduction in maternal anemia and placental parasitemia. In multigravidae, IPTp was associated with a reduction in LBW. During this time period, IPTp remained beneficial in this area of western Kenya, despite high SP resistance.

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ORIGIN OF PLACENTAL MALARIA INFECTION AND RESPONSE TO TREATMENT DURING PREGNANCY

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Placental malaria is a significant cause of maternal anemia and infant low birth weight. Little is known about the characteristics of peripheral malaria infections during pregnancy that lead to placental infections. We sought to determine when during pregnancy peripheral infection leads to placental sequestration and whether sulfadoxine-pyrimethamine intermittent preventive treatment (SP-IPT) or lumefantrine-artemether (LA) treatment clear the parasites sequestered in the placenta. We screened 325 placentas from women enrolled in an observational study of malaria during pregnancy. We used 6 neutral microsatellite markers to genotype placental and peripheral parasites. Placental parasites from 17 women

were fully genotyped. Mean gestational age (GA) at enrollment was 18 weeks (range 13-24). Of 39 visits with any peripheral parastemia, 25 (65%) were sub-microscopic. Four of 17 women with molecular evidence of placental malaria did not experience any peripheral parasitemia during follow-up. Among the 13 women with peripheral parasitemia during follow-up, 6 (46%) had peripheral genotypes matching placental genotypes. Matching genotypes occurred later in pregnancy than did non-matching (34 weeks vs. 25 weeks). SP-IPT cleared peripheral parasitemia in 4 of 8 (50%) cases and LA cleared peripheral parasitemia in 7 of 7 cases. Recrudescence after treatment with LA occurred after 3 of 7 doses at 25, 45, and 78 days after treatment. These data suggest the majority of women experience the peripheral infection leading to placental infection prior to 18 weeks GA and the majority of peripheral infections experienced by women who went on to have placental malaria were submicroscopic infections. LA, but not SP-IPT, cleared all peripheral infections. Both SP-IPT and LA treatment allowed for recrudescence of parasites during pregnancy, which likely reflects their failure to eliminate parasites sequestered in the placenta.

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ASSESSING MALARIA SURVEILLANCE DATA QUALITY: EXPERIENCE FROM BENIN, ETHIOPIA AND UGANDA

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Health facility-based malaria surveillance in Africa presents challenges due to reporting based on clinical diagnosis but lacking diagnostic confirmation. However, the scale up of rapid diagnostic tests and shifting national policies to universal testing may alleviate many of these challenges. We evaluated three models of malaria sentinel surveillance in Benin, Ethiopia, and Uganda to identify the unique attributes of each system and evaluate standard metrics of data quality. Compared to routine health facility data, Benin's system provided complete and comprehensive malaria data and filled an important data gap for the national program. In Ethiopia, a multi-tiered reporting system leveraged an existing network of health extension workers for monitoring malaria cases in the community. This system provided epidemic detection for entire health facility catchment areas. In Uganda, ongoing supervision provided by the implementing partner resulted in strengthened malaria diagnostic capacity and a high testing proportion of suspect cases. In all three countries, key performance indicators were high: completeness of malaria indicators was >95%; accuracy was >75%; and the average proportion of suspect cases tested was >75%. Timeliness of monthly reporting was satisfactory for all systems but epidemic detection would be strengthened by more frequent reporting. Results of analyses in all three countries showed that system performance improved with frequent supervision, clear standard operating procedures, a laboratory quality control system, and simple data collection tools. Data use by health workers resulted in greater compliance to reporting procedures and better data quality. In all countries sentinel surveillance data was of superior quality compared to routine system data. Our results suggest that near universal testing and improved data quality exemplified by these three surveillance systems with distinctly different implementation have improved the usefulness and public health impact of malaria surveillance data.

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RELIABILITY OF SCHOOL SURVEYS IN ESTIMATING GEOGRAPHIC VARIATION IN MALARIA TRANSMISSION IN THE WESTERN KENYAN HIGHLANDS

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To evaluate the effectiveness of control interventions against malaria, reliable estimates of malaria transmission within the community are essential. Cross-sectional surveys can be logistically demanding and prohibitively expensive for control programs if required repeatedly. Health facility data, whilst less expensive and logistically simpler, often rely on clinically diagnosed malaria so are therefore likely to miss asymptomatics and will be affected by health-seeking behavior. An alternative approach is to use school surveys, which are increasingly being used for estimating disease prevalence and may act as a focal point for rolling out interventions. Here we carried out surveys in primary schools in Rachuonyo South district in the highlands of western Kenya in July 2010 at the same time as cross-sectional surveys within the immediate community to compare prevalence of malaria by rapid diagnostic tests (RDT) and antibody responses to the *P.falciparum* merozoite antigen MSP-1_{19} and AMA. All results obtained at the school were geolocated by following up children to their homes. Crude RDT prevalence from school data was 24% whilst that recorded from community surveys was 16%. Comparing RDT prevalences between school-level data and community surveys, resulted in a correlation coefficient of 0.74, with 42% of the community results being significantly different to those obtained at the school. This increased to a correlation coefficient of 0.81 when data within the community was restricted to school-age children. For this subset of data, only 13% of the paired school and community prevalence estimates were significantly different. Factors determining these differences focusing on altitude, distance of pupil households from the school and use of malaria control interventions will be presented. This data will be supplemented with age specific sero-prevalences and estimates of the sero-conversion rates. The utility of school-based sampling using RDT results and serology to discriminate areas of high and low transmission will be discussed.

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ASSESSMENT OF MALARIA CONTROL PROGRESS OVER A TWO-YEAR PERIOD USING A CONTINUOUS 'ROLLING' MALARIA INDICATOR SURVEY ACROSS AGE GROUPS IN CHIKHWAWA DISTRICT, MALAWI

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Low cost, district-level monitoring and evaluation (M&E) tools that can provide real-time malaria control progress are urgently needed to guide and optimize control efforts and impact. From May 2011 we have conducted a continuous 'rolling' Malaria Indicator Survey (rMIS) in children aged 6-59 months in 51 villages within Chikhwawa district, Southern Malawi. In 2011, district wide indoor-residual spraying and the use of Rapid DiagnosticTests were added to facility-based ACT case-management and the distribution of insecticide treated bednets. Monthly collection of standard malaria intervention coverage and burden indicators were conducted by a small team of 2 nurses and 2 field workers, sampling all villages twice a year, using PDAs for data capture. Findings from the first

year identified substantial temporal and spatial variation in intervention coverage and malaria transmission within the area. The continuous rMIS approach provided real-time feedback on coverage gaps and burden hotspots, suggesting that this type of M&E surveys would become an intervention in itself if could trigger specific local focused control action, and could strengthen our current arsenal of interventions. With the increasing focus on universal coverage and transmission reduction, the rMIS was expanded to include older children and adults in the second year (June 2011-May2012). Preliminary results of this second year rMIS will be presented, with a focus on the added value of including older age groups in MIS surveys and control progress over both years.

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BILHARZIA IN THE INFORMAL URBAN SETTLEMENTS OF WESTERN KENYA: PREVALENCE, DISTRIBUTION AND EVALUATION OF COMMUNITY AND SCHOOL-BASED APPROACHES FOR CONTROL

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Urban areas present unique challenges for primary health care, which have remained poorly researched, and urban bilharzia remains a neglected area when prioritizing intervention strategies. Control of schistosomiasis and soil-transmitted helminthiasis is hampered by poverty, inadequate clean water, occupational hazards and poor sanitation. The cross-sectional study determined the prevalence and distribution of schistosome and soil-transmitted helminth (STH) infections, among 1,308 children in 34 primary schools and in intermediate snail vectors in 8 informal urban settlements in Kisumu City. Schools, water bodies and snails were mapped and fecal contamination (presence of Escherichia coli) of public water sources determined. Community health workers, village elders, and teachers were sensitized on Bilharzia and trained on mass drug administration (praziquantel) to community members or school going children respectively. Prevalence of Bilharzia was 36% in one of the informal settlement areas (Nyalenda B) and over 10% in all other informal settlements. The overall prevalence for STHs was 16%. Of the snails collected, 1.8% shed schistosome cercariae and 95% of water sources sampled were contaminated with fecal matter. In the MDAs, about 60% of the target population was treated by CHWs in the community while the school-based treatment achieved over 90% coverage.This study observed that schistosomiasis and STH are important health priorities among schools in informal settlements of Kisumu City. The study confirmed that besides L. Victoria, schistosomiasis transmission exists within the informal settlements of Kisumu City. Snail control, treatment of public water sources and improvements in local sanitation and public health awareness are advocated for in such settings.

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PREDICTIVE VALUE OF SCHOOL AGE CHILDREN'S SCHISTOSOMIASIS PREVALENCE FOR PREVALENCE IN OTHER AGE GROUPS AND THE EFFECT OF ONE ROUND OF SCHOOL-BASED OR COMMUNITY-WIDE TREATMENT IN WESTERN KENYA - THE SCORE PROJECT

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With increased global commitment to schistosomiasis control, mass drug administration (MDA) programs are being implemented in several settings. Depending on the prevalence of infection in school age children within a given community, WHO recommends that either school-based or community-wide MDA be employed. To test the assumption that school prevalence reflects the underlying community prevalence, we evaluated how well infection prevalence and intensity in 9-12 year old school pupils correlated with infection levels in other children and adults within the same community. Cross-sectional surveys of pre-adolescents (9-12 years old) were compared to those of first year students (7-8 years old), adolescents (13-14 years old) and adults (20-55 years old) in 150 villages along the shores of Lake Victoria. Written informed consent was obtained from adults and both consent and assent were obtained for children. A single stool sample was collected from 50 adults, 50 adolescents and 100 first year students and three stools were collected from 100 pre-adolescents in each village. Two slides per stool were screened for *Schistosoma mansoni* using the Kato Katz method. Data were analyzed using Spearman's nonparametric correlation analysis; p values < 0.05 were considered significant. We surveyed 3900 first year students, 12037 pre-adolescents, 5417 adolescents and 7566 adults. Of these, 1098 (28.2%) first year students, 7390 (61.4%) pre-adolescents, 2207 (40.7%) adolescents, and 3185 (42.1%) adults were positive for *S. mansoni* infection. Initial evaluation suggested that a village's schistosomiasis prevalence for 9-12 years olds significantly correlated with prevalence for all other age groups, suggesting that this age group is in fact a good predictor. Preliminary analysis of infection levels in the 9-12 year old age group one year following MDA suggests that children in villages randomized to the school-based treatment arms had lower prevalence and intensity of infection than children in villages randomized to the community-wide treatment arms.

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COMPARING THE COST OF SCHOOL-BASED VERSUS COMMUNITY-WIDE PRAZIQUANTEL MASS DRUG ADMINISTRATION IN KENYA

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In addition to impact on burden of schistosomiasis, cost can be an important factor in mass drug administration (MDA) program design. The Schistosomiasis Consortium for Operational Research and Evaluation (SCORE) is conducting studies in several African countries that analyze the benefits and costs of implementing either school-based or community-wide treatments over a 4-year intervention period. As part of the cost benefit analysis, cost estimates of the second year of MDA in SCORE projects in Kenya were calculated. Twenty-four out of 175 villages were selected for the study based on distance, school size, district, and initial schistosomiasis prevalence, including 8 villages with 10-25% initial prevalence and 16 with > 25% initial prevalence. Information about various inputs and resources used to conduct MDA were collected from the transportation department, human resources department, study coordinators, field coordinators, and project associates. Costs that varied across villages were identified and costs that were consistent across villages were determined a priori. Each cost was associated with an MDA activity such as advocacy, mobilization, drug distribution, coverage or feedback. Preliminary data analysis suggests that school based MDA costs less than community wide treatment. The major drivers of cost associated with community wide MDA were transportation and personnel costs. In contrast school based distribution of treatment was centralized and five days of salary for community distributors was not required. The final Kenya MDA cost effectiveness analysis will include impact of treatment, either school-based or community-wide, on disease prevalence after 4 years of intervention in order to describe the relationship between the cost of alternative MDA approaches and the benefits achieved in terms of decreases in prevalence and intensity of schistosomiasis.

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EVALUATION OF THE HEALTH-RELATED QUALITY OF LIFE (HRQOL) OF CHILDREN IN A *SCHISTOSOMA HAEMATOBIUM*-ENDEMIC AREA IN KENYA

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Schistosomiasis remains a public health challenge; 93% of the estimated 237 million infections occur in sub-Saharan Africa. Though rarely fatal, its recurring nature makes it a lifetime chronic disorder with significant health burden. Much of its negative health impact is due to subtle conditions such as anemia, undernutrition, pain, exercise intolerance, poor school performance, and decreased work capacity. This makes it difficult to estimate the disease burden specific to schistosomiasis using the current DALY metric. In our study, we used Pediatric Quality of Life Inventory (PedsQL), a modular instrument available for a wide range of ages (2-18 y), to assess Health-related Quality-of-Life (HrQoL) in children living in a S. haematobium-endemic area in coastal Kenya. The PedsQL questionnaires were administered by interview to children aged 5-18 y (and their parents) in 5 villages spread across three districts. HrQoL (total score) was significantly lower in villages with high prevalence of S. haematobium (-4.0 + 0.8%, $p<0.001$) and among the lower socioeconomic quintiles (-2.0 + 0.8%, $p<0.01$) after adjustment for age, sex, and undernutrition. A greater effect was seen in the psychosocial scales as compared to physical function scale. Individual S. haematobium egg output was not associated with PedsQL score within the subset of three high-prevalence villages, whereas, in low prevalence villages, detection of any eggs in the urine were associated with a significant -2.1 + 0.9% ($p=0.025$) reduction in total score. The PedsQL reliabilities were high (Cronbach alphas generally ≥0.70), floor effects were acceptable, and identification of children from low socioeconomic status was valid. We conclude that urogenital schistosomiasis is specifically associated with at least a 2-4% reduction in HrQoL. Further research is needed on reproducibility and responsiveness properties of QoL testing in relation to schistosomiasis; we expect that a case definition based on more sensitive diagnosis will better define the immediate and long-term QoL impact of S. haematobium infection.

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COMMUNITY PERCEPTIONS OF SCHISTOSOMIASIS RISK AMONG SCHOOL CHILDREN IN ZANZIBAR

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School-aged children on Unguja and Pemba Islands (Zanzibar) are at particular risk of infection by *Schistosoma* haematobium, a schistosome species that causes urinary schistosomiasis, a neglected tropical disease common throughout much of Africa. Despite the high prevalence of schistosomiasis (locally called Kichocho) in some communities, little is known about the community's perspectives on the disease among school children. In 2011, as part of a larger study aiming for schistosomiasis elimination, qualitative data were collected in Zanzibar from 39 groups of children, 45 community leaders, 21 teachers and 16 parents to better understand their knowledge, perceptions and practices associated with preventing, controlling, and treating Kichocho in children. Using a grounded theory approach, we transcribed, coded, and analyzed the data. Kichocho was not seen as a disease of females. People typically acquired their knowledge through informal social networks and characterized the disease as one of young boys spending time in a dirty pond or stream. Identification of the parasite and mode of transmission was lacking. People often failed to seek treatment for children due to anticipated costs and home treated with plant-based teas and water. Schools lacked Kichocho education curriculums. People recognized the need for prevention and suggested organizing educational trainings for public and religious schools and the community; developing interactive teaching tools; partnering with student clubs to educate students; working with the community to build latrines, urinals, wells, and washing platforms near the river and at home; building play areas and offering play opportunities for children; and providing free local drugs. Our findings illuminated major gaps in local knowledge as well as practical, structural, educational, cultural and medical issues to consider when preparing for mass drug distribution and school-based interventions as well as the need to collaborate with the community on future prevention efforts.

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MEFLOQUINE-PRAZIQUANTEL FOR THE TREATMENT OF *SCHISTOSOMA* HAEMATOBIUM INFECTIONS IN SCHOOL-AGED CHILDREN IN CÔTE D'IVOIRE

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The global strategy for schistosomiasis control is morbidity control, relying on a single drug, praziquantel. Although no clinically relevant resistance to praziquantel has been described to date, development of drug resistance is of growing concern as control efforts are going to scale. We have recently shown that mefloquine possesses promising antischistosomal properties *in vitro*, *in vivo*, and in proof-of concept clinical trials. In contrast to praziquantel, high worm burden reductions were observed following mefloquine treatment in the juvenile *Schistosoma* mansoni infection mouse model. Additionally, synergistic interactions were observed *in vitro* and in the S. mansoni-mouse model, when praziquantel was combined with mefloquine. We present results from the first exploratory randomized trial in school-aged children in southern Côte d'Ivoire evaluating the efficacy and safety of mefloquine (25 mg/kg) combined with praziquantel (40 mg/kg), and mefloquine/artesunate (3 x (100 mg artesunate + 250 mg mefloquine) combined with praziquantel (40 mg/kg) compared to standard praziquantel treatment (40 mg/kg) against S. haematobium. In the absence of prior drug interaction studies, drugs were administered on subsequent days. Two urine samples were collected before and on days 21-22 and 78-79 after the first dosing. Sixty children were present on all examination time points. No significant difference in efficacy was observed between the three treatment groups on the first treatment follow-up (mefloquine-praziquantel: cure rate (CR), 32%, egg reduction rate (ERR), 95%; mefloquine-artesunate-praziquantel: CR, 32%, ERR 95%; praziquantel: CR, 30%, ERR, 93%) and on days 78-79 posttreament (mefloquine-praziquantel: CR, 32%, ERR, 94%; mefloquine-artesunate-praziquantel: CR, 33%, ERR, 92%; praziquantel: CR, 19%, ERR, 93%). Adverse events were mostly mild in all treatment groups. In conclusion, the addition of mefloquine or mefloquine-artesunate does not enhance the efficacy of praziquantel in the treatment of S. haematobium.

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PREDICTIVE MAPPING VS. EMPIRIC ASSESSMENT OF SCHISTOSOMIASIS: IMPLICATIONS FOR TREATMENT PROJECTIONS IN GHANA

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Mapping the distribution of schistosomiasis is essential to determine where control programs should operate, but because it is impractical to assess infection prevalence in every potentially endemic community, model-based geostatistics (MBG) is increasingly being used to predict prevalence and determine intervention strategies. To assess the accuracy of MBG predictions for *Schistosoma hematobium* infection in Ghana, school surveys were evaluated at 79 sites to yield empiric prevalence values that could be compared with values derived from recently published MBG predictions. Based on these findings schools were categorized according to WHO guidelines so that practical implications of any differences could be determined. Using the predicted values alone, 21 of the 25 empirically determined 'high-risk' schools requiring yearly praziquantel would have been *undertreated* and almost 20% of the remaining schools would have been treated despite empirically-determined absence of infection - translating into 28% of the children in the 79 schools being undertreated and 12% receiving treatment in the absence of any demonstrated need. Using the current predictive map for Ghana by aggregating prevalence estimates to the district level was clearly not adequate for guiding the national program, but the alternative of assessing each school in potentially endemic areas of Ghana or elsewhere is not at all feasible; modelling must be a tool complementary to empiric assessments. We conclude that for practical usefulness, predictive risk mapping should not be thought of as a one-time exercise but must, as in the current study, be an iterative process that incorporates empiric testing and model refining to create updated versions with increasingly accurate predictions.

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INTERVENTIONS TO STABILIZE ENDOTHELIUM IMPROVE SURVIVAL IN EXPERIMENTAL CEREBRAL MALARIA

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Cerebral malaria (CM) pathogenesis is associated with endothelial activation and perturbation of the blood brain barrier (BBB). Endothelial specific signalling pathways, including the Angiopoietin (Ang)-Tie-2 and Slit/ Roundabout (Robo)-4 systems, are key regulators of endothelial integrity and vascular leakage. Our lab and others have reported that adult and pediatric CM is associated with increased circulating levels of biomarkers of endothelial activation and dysfunction (e.g. Ang-2, sTie-2, sICAM-1). We hypothesize that interventions to promote endothelial stability will prevent deleterious alterations to the BBB and improve outcome following *Plasmodium* infection. Using the murine model of *Plasmodium berghei* ANKA (PbA)-induced experimental CM (ECM), we show that alterations in protein and mRNA levels of angiopoietins are associated with disease severity in the ECM, similar to observations in human populations. Time course experiments established a temporal relationship where PbA-associated alterations in endothelial regulators directly precede the loss of BBB integrity and the onset of neurological symptoms of ECM, such as seizures and paralysis. Pro-Ang-1 treatment strategies (e.g. Adenoviral mediated expression of Ang-1) significantly improved survival in PbA-infected ECM-susceptible C57Bl/6 mice compared to empty adenoviral vector and vehicle controls ($p=0.001$). Pharmacological activation of the Slit-Robo pathway, using therapeutic administration of recombinant Slit2N, also significantly prolonged survival in PbA-infected C57Bl/6 mice compared to untreated controls ($p=0.0007$). This benefit was further increased when Slit2N was used as adjunctive therapy in combination with a sub-curative dose of artesunate. To establish direct experimental evidence for a causal role of angiopoietins in ECM, the effect of Ang-1 genetic deletion on disease outcome is currently under investigation using a conditional Cre/loxP system. In summary, we show that adjunctive treatment strategies based on promoting endothelial quiescence and BBB integrity improve survival in ECM.

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POSITRON EMISSION TOMOGRAPHY - AN *IN VIVO* IMAGING SYSTEM FOR FOLLOW UP ENCEPHALIC METABOLISM IN CEREBRAL MALARIA

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Cerebral malaria (CM) is a neurological manifestation of *Plasmodium falciparum* infection which accounts for over 1 million deaths per year worldwide. About 25% of CM patients develop neurocognitive deficits, including memory loss and speech and learning impediments. As proper brain metabolism is critical to neurocognition, it may be altered in CM, but its role is poorly characterized. In addition, human CM studies are restricted to postmortem observations thus limiting our ability to characterize brain metabolic activity during disease. Non-invasive *in vivo* diagnostic tools are therefore needed to monitor the progression of CM. To investigate cerebral metabolic alterations in murine CM, we used positron emission tomography (PET) to monitor radioactive concentrations of fluorodeoxyglucose (FDG), a glucose analogue which reflects tissular metabolic activity. We examined encephalic metabolic activity in uninfected C57BL/6 mice and mice infected with *Plasmodium berghei* ANKA (PbA), a mouse malarial strain which causes CM. Throughout the course of disease, glucose uptake was decreased in several brain regions in PbA-infected mice compared to controls, including the cerebral cortex, olfactory bulb, brainstem and cerebellum. There was also a significant effect of infection and time on mean expression of FDG in the eyes, indicating an ocular decrease of metabolism, which might be correlated to the known retinopathy of the disease. More importantly, decreased glucose uptake correlated temporally with increased CM pathology, thereby establishing a new tool to study disease. With FDG-PET, we have come up with a novel imaging tool to non-invasively study brain metabolism during CM. FDG-PET-CT will serve as an unprecedented translational technique to understand the brain metabolism in human CM patients.

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SCHISTOSOMA MANSONI POLO-LIKE KINASES : KEY REGULATORS OF REPRODUCTIVE ORGAN DEVELOPMENT

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Polo-like kinases (Plks) constitute a family of conserved serine/threonine protein kinases known as important regulators of cell cycle progression and mitosis. Yeasts have only one Plk whereas vertebrate species possess five Plks (Plk1-5). Plk1, homolog to the *Drosophila* kinase Polo, is the best characterized member of the Plk family. Plk1 plays a major role in cell cycle progression by triggering G2/M transition and since it is overexpressed in various cancers, Plk1 constitutes a valuable target for anti-cancer therapy. Plk4/Sak (Snk akin kinase) is a divergent member of the family, structurally distinct from other Plk members, with essential functions in centriole duplication. The trematode parasite *Schistosoma mansoni*, responsible for schistosomiasis, has only, like *Drosophila*, two Plks, SmPlk1 and SmSak. Transmission and pathogenesis of schistosomiasis is due to the exceptional fecundity of schistosomes, for which Plks has been shown to play a decisive role. Both transcripts for schistosome Plks have been localized specifically in reproductive organs of female and male worms, with a majority of *SmSak* in ovary. Moreover, the treatment of worms with BI2536 (the anti-cancer drug inhibiting specifically Plk1 and SmPlk1) has shown a key role of SmPlk1 in gametogenesis and parasite reproduction, emphasizing its potential use as a novel therapeutic target against schistosomiasis. Studies in *Xenopus* oocyte, used as a protein expression system, have shown that the respective role of SmPlk1 and SmSak in G2/M transition triggering and centriole duplication during the cell cycle progression. Moreover, in these experiments, an unexpected interaction was demonstrated between SmPlk1 and SmSak, that could lead to Plk activation and spontaneous meiosis resumption in Plx1-depleted oocytes. These results suggest that Plk1 and Plk4 proteins are susceptible to interact and cross-activate in cells and thus attribute for the first time a potential role of Plk4 proteins in meiosis/mitosis entry. In addition to SmPlk1, this unexpected role of SmSak in meiosis could be relevant to further consider the function of this novel Plk in schistosome reproduction.

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UBIQUITIN FOLD MODIFIER (UFM-1) PROTEIN IS AN *L. MEXICANA* VIRULENCE FACTOR WHICH CONTRIBUTES TO PATHOGENESIS IN CL

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In this study, we generated *Leishmania mexicana* (Lm) lacking ubiquitin fold modifier (Ufm-1) gene, and examined *in vitro* parasite survival in dendritic cells and virulence *in vivo* using a murine model of CL. We found efficient internalization of both WT and Ufm-1-/- parasites by bone marrow derived dendritic cells (DCs), although Ufm-1-/- parasites were cleared significantly faster than WT parasites by DCs. We also found that Ufm-1-/- *Lm*-infected DCs produce significantly less IL-10 compared to WT *Lm*-infected DCs upon LPS stimulation *in vitro*. BALB/c mice infected with WT *L. mexicana* developed large non-healing lesions while Ufm-1-/- *Lm*-infected BALB/c mice had delayed lesion growth and developed smaller lesions by week 10 post-infection. Analysis of *Leishmania*-specific serum antibodies revealed that WT infected mice produced significantly higher titers of *Lm*-specific Th2-associated IgG1 than Ufm-1-/- *Lm*-infected mice, although *Lm*-specific IgG2a production was undetectable in both groups. Upon *in vitro* stimulation with *Lm* antigen, draining lymph node cells from WT *Lm*-infected mice produced significantly more IL-4 compared to similarly stimulated cells from Ufm-1-/- *Lm*-infected mice although IFN-γ production was comparable between the two groups. Taken together, our findings show that Ufm-1 is a *L. mexicana* virulence factor which contributes to establishment of infection and pathogenesis in CL. Furthermore, we demonstrate that Ufm-1 is not essential for parasite survival.

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THE EXISTENCE OF A G1 CELL CYCLE CHECKPOINT IN *P. FALCIPARUM* MEDIATED BY THE CYCLIN-DEPENDENT PROTEIN KINASE PFMRK; IMPLICATIONS FOR COMPOUND SELECTION AND INHIBITORY GROWTH ASSAY DEVELOPMENT

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Rapid growth and multiplication of *Plasmodium falciparum* during erythrocytic schizogony result in clinical symptoms and disease progression of malaria. Parasite growth is controlled by an unknown cell cycle regulatory mechanism, but believed to be similar to that of mammalian cells. However, there are many features of parasite schizogony that are unique. The ring stage of *P. falciparum* is representative of the G1 phase, while trophozoite and schizont stages are equivalent to S and M phases respectively. The regulation of how the parasite transits through these cell cycle phases and whether cell cycle checkpoints exist are unknown. Cyclin-dependent protein kinases (CDKs) are essential regulators for sequential growth and proliferation. Pfmrk, a sequence homologue of human CDK7 is suggested to play a role in both cell cycle control and DNA replication in *P. falciparum*. Transgenic parasites that over-express functional Pfmrk (HPG), non-functional Pfmrk (HKG), or control (empty vector control), revealed that HKG parasites exhibited a delay in the completion of the intraerythrocytic development cycle. To investigate the role of cell cycle regulators in *Plasmodium* growth and development, we assessed 41 mammalian cell cycle inhibitors, such as CDK inhibitors, DNA synthesis inhibitors and mitotic inhibitors, for growth inhibition. Of these compounds, 8 that significantly inhibited parasite growth (IC_{50} < 10 μM) were shortlisted for further studies. FACS analysis demonstrated that control parasites treated with kenpaullone, a G1/S mammalian cell cycle inhibitor that inhibits Pfmrk kinase activity, "arrested" at trophozoite stages, whereas HPG parasites treated with the same inhibitor transitioned sooner from trophozoites to schizonts. In stage-specific growth inhibition studies, HPG parasites treated at trophozoite-stage were less sensitive to the growth inhibitory effects compared to early ring-staged treatment. Moreover, HPG parasites treated at early ring-stage development indicated a delay in the initiation of the next growth cycle by approximately five hours. The results suggest Pfmrk functions at the ring-trophozoite transition, reminiscent of a G1 checkpoint. The existence of a checkpoint would have a profound effect on the selectivity of compounds and warrant consideration for how and when compounds are tested in growth inhibition assays.

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INVASION GENE HAPLOTYPES ASSOCIATE WITH PARASITEMIA IN HUMANS REPORTING WITH *PLASMODIUM KNOWLESI* MALARIA IN MALAYSIAN BORNEO

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Human infections with *Plasmodium* knowlesi, a parasite of long and pig-tailed macaques, continue to be reported in most countries within Southeast Asia. Parasite invasion occurs daily in P. knowlesi infections and parasitemia is associated with disease severity. In this study we test the hypothesis that parasitemia is associated with particular alleles of two genes, P. knowlesi normocyte binding protein xa and xb (Pknbpxa and Pknbpxb), encoding invasion proteins on the merozoite apex. In the first instance a fragment 8500bp beginning at exon II of the Pknbpxa gene and a fragment 3500bp beginning at Exon I of the Pknbpxb gene were cloned and sequenced to high stringency using 5 reference isolates collected at geographically distinct locations. Sequence alignment indicated that most diversity occurred at the 5' region of exon II for both genes. Fragments with 37 non-synomymous and 7 synonymous substitutions with nucleotide diversity (ϖ) = 0.024 of Pknbpxa and 14 non-synonymous and 2 synonymous substitutions, ϖ=0.0056 of Pknbpxb were chosen to haplotype 147 P. knowlesi isolates from clinically well-characterised patients. Pknbpxa haplotypes were obtained for 138 isolates, 7 failed to amplify and 2 failed to sequence. Pknbpxb haplotypes were obtained for 134 isolates, 3 patients had multiple genotype infections and were excluded and 10 isolates failed to amplify. Within the Pknbpxa haplotyping fragment there were 82 polymorphic sites (56 non-synonymous, 26 synonymous substitutions) ϖ = 0.02269 and 47 polymorphic sites (28 non-synonymous and 19 synonymous substitutions) ϖ = 0.00642 within the Pknbpxb fragment. There were 75 Pknbpxa and 51 Pknbpxb haplotypes in the study population with haplotype diversity (h) of 0.9729 and 0.9216 respectively, suggesting high polymorphism among the isolates. Non-synonymous single nucleotide polymorphisms(SNPs), where the minor allele was represented in >10% of the isolates, were analysed for association with parasitemia. Preliminary analyses found significant associations between two Pknbpxa and one Pknbpxb SNPs and parasitemia suggesting that particular alleles may influence erythrocyte invasion efficiency in human infections. The results of this study will be presented within the context of parasitemia and disease severity in P. knowlesi malaria.

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INVASION GENE HAPLOTYPES ASSOCIATE WITH PARASITEMIA IN HUMANS REPORTING WITH *PLASMODIUM KNOWLESI* MALARIA IN MALAYSIAN BORNEO

Mohammed Atique Ahmed[1], Balbir Singh[1], Ing Tien Wong[2], Chan Woon Lu[3], Ramlah bt Zainudin[1], Paul C.S. Divis[1], David J. Conway[4], Sanjeev Krishna[5], Janet Cox-Singh[5]

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1455

PHASE I TRIAL OF PFS25-EPA/ALHYDROGEL® A TRANSMISSION BLOCKING VACCINE AGAINST *FALCIPARUM* MALARIA IN HEALTHY MALARIA-NAÏVE ADULTS

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We describe the results of a phase I dose-escalating clinical trial to assess the safety and immunogenicity of the transmission blocking vaccine Pfs25-EPA/Alhydrogel®. Pfs25 has previously been shown to induce antibody which inhibits parasite development in a standard membrane feeding assay (SMFA), but an immunogenic formulation safe for human use has been lacking. EPA as a conjugate has been shown to enhance immunogenicity and to be safe in humans. Pfs25 and EPA were chemically conjugated and adjuvanted with Alhydrogel. 30 subjects have received up to three doses of 8, 16 (at 0 and 2 months) or 47-µg of Pfs25 at 0, 2 and 4 months. Vaccinations were generally well tolerated. The majority of solicited adverse events were mild in severity. No vaccine related serious adverse events occurred. The most common solicited adverse event was pain at the injection site, and the frequency of adverse events decreased with each successive dose of vaccine. A decrease in hemoglobin was seen in 8 of 30 subjects after the first vaccination, in 11 of 26 subjects after the second and 2 of 15 after the third vaccination. The majority of these were mild in nature, a few were moderate, and most were in subjects who had a previous history of low hemoglobins or anemia and were judged to be unrelated to vaccination. The vaccine was more immunogenic with each successive dose. Geometric mean antibody levels in the 47 µg dose group

were 92 EU (95% CI 55, 155) and 228 EU (95% CI 151, 344) after the second and third vaccinations respectively. Sixteen of 17 subjects in the 47 µg dose group had detectable antibody response after 2 vaccinations; 15/15 had responses after 3 vaccinations. Transmission blocking activity correlates with antibody titer, as demonstrated by SMFA. The data to date demonstrate that Pfs25-EPA/Alhydrogel® is well tolerated, increasingly immunogenic with each dose, and induces antibodies which inhibit parasite development in the mosquito.

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COMPARATIVE ASSESSMENT OF TRANSMISSION BLOCKING MALARIA VACCINE CANDIDATE ANTIGENS USING AN ADENOVIRUS-MVA PRIME-BOOST REGIME

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Transmission blocking vaccines (TBVs) target *Plasmodium falciparum* sexual stages, aiming to block development within the mosquito. Different delivery systems, mainly protein-in-adjuvant formulations, have been previously employed giving varied transmission blocking activity (TBA). However, leading TBV candidate antigens have not been comparatively assessed to determine a rank order of their TBA. Simian adenovirus (ChAd63) and Modified Vaccinia Ankara (MVA) in a prime-boost regime were used to induce antibodies against five candidate antigens and assessed their TBA. Antigen sequences were codon optimised and cloned into ChAd63 and MVA to generate recombinant viral vectored vaccines. These were used to vaccinate Balb/c mice in a 70 day regimen comprising of a day 0 ChAd63 prime and MVA boost at day 56. Antibody responses were measured days 14, 55 post-prime and day 70 post-boost by ELISA. TBA against *P. falciparum* NF54 strain and African field isolates was assessed by SMFA using purified IgG from sera taken at day 70. Antibody responses measured provided evidence that the antigens were immunogenic with ChAd63 priming responses boosted following MVA vaccination. TBA exhibited against *P. falciparum* NF54 in *Anopheles* stephensi ranged between 16-100% giving a rank order of the antigen-specific antibody's ability to inhibit oocyst intensity. This rank order was replicated against field *P. falciparum* isolates from gametocyte carriers in *Anopheles gambiae*. Hence the antigens with the highest TBA (90-100%) were further tested at varying IgG concentrations giving 39-100% efficacy. Two out of the five antigens consistently showed 99-100% with 0-5% infectivity to the mosquito. Antibodies induced by viral vectors showed partial to complete blockade depending on the target antigen. This antigen delivery system provides a robust vaccine platform for inducing antibodies against target antigens and has enabled a head-to-head comparison of TBV candidates. This comparative analysis is essential to guide and inform future assessment of candidates for clinical development.

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FUNCTIONAL COMPARISON OF LEADING *PLASMODIUM FALCIPARUM* TRANSMISSION BLOCKING VACCINE CANDIDATES BY STANDARD MEMBRANE FEEDING ASSAY

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Recently, there has been a renewed interest in the development of vaccines against the sexual stages of *P. falciparum* malaria. While several potential transmission blocking vaccine (TBV) candidates have been reported, studies directly comparing them in a functional assay are limited. To this end, recombinant proteins of 5 leading TBV candidates, Pfs25, Pfs48/45, Pfs230, PfHAP2, AnAPN1, and GST (as a control) were expressed in the wheat germ cell-free expression system. CD-1 outbred mice (n=10 per group) were immunized twice with the antigens adjuvanted with Montanide ISA720. Two weeks after the boost, antibody levels were measured by ELISA and the functionality of antibodies was assessed by a standard membrane feeding assay (SMFA) using cultured *P. falciparum* NF54 gametocytes and *Anopheles* stephensi mosquitoes. The levels of antibodies for all antigens were relatively similar (33,000 to 88,000 ELISA units as a median). For the functional analysis we prepared a pool of serum from each group and isolated IgGs from each by Protein G purification. The purified IgGs were tested at 0.75 mg/ml (the concentration at which mouse IgGs have shown minimum non-specific inhibition) by SMFA. Anti-Pfs25, anti-Pfs230 and anti-PfHAP2 antibodies showed 97-100% inhibition in oocyst density compared to anti-GST antibody, and these inhibitions were all statistically significant ($p<0.01$, Kruskal-Wallis test followed by Dunn's multiple comparison test). We confirmed the inhibitory activity of these three antibodies in an independent assay (93-100% inhibition in the second test), and the inhibition was dose-dependent. Alternatively, anti-Pfs48/45 (-48% inhibition) and anti-AnAPN1 (-11% inhibition) antibodies did not show any inhibition at 0.75 mg/ml. Of these 5 antigens expressed in the wheat germ cell-free expression system, antibodies to Pfs25, Pfs230 and PfHAP2 proteins showed superior functional activity in this study. Further studies of these 3 products are in progress and the current SMFA results support future TBV development of the candidates produced in this system.

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EFFICACY OF TRANSMISSION BLOCKING VACCINE CANDIDATES IN BURKINA FASO

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Malaria parasite transmission from humans to mosquitoes requires the ingestion of gametocytes that circulate in the human blood by *Anopheles* mosquito vector followed by several steps of parasite development in the mosquito. Transmission-blocking vaccines aim at impeding parasite development in the vectors and are nowadays viewed as a promising strategy for breaking this transmission and an important component for achieving malaria elimination and eradication. Antibodies specific for the vaccine candidate antigens Pfs25 and Pfs230 showed efficacy to limit *Plasmodium falciparum* transmission to mosquitoes in laboratory conditions. In the present study we aimed at assessing their efficacy against field isolates of parasites from Burkina Faso in semi natural conditions of transmission. Standard Membrane Feeding Assays (SMFA)

were carried out by exposing An. gambiae s.s females to gametocyte-infected blood from naturally infected patients. Pfs25 and Pfs230 antibodies, produced by mice immunization using recombinant viral vectors, were tested at different concentrations added to the blood, using different gametocytes densities. In parallel, an entomological study was performed in order to assess the natural parasite load in local mosquito vectors. SMFA revealed 100% transmission blocking activity (TBA) for both antibodies at titer from 62.5 to 500µg/ml, depending on infection intensity in the control mosquito group. Field collections showed that among the 2,293 wild mosquitoes dissected, 275 carried oocysts with an average of 8 oocysts per infected mosquito. For such a parasite load, we observed that a concentration of 250µg/ml of either Pfs25 or Pfs230 antibodies has a complete TBA activity. Our results demonstrated that Pfs25 and Pfs230 antibodies strongly limit human to mosquito *P. falciparum* transmission, suggesting that these antigens are valuable candidates for transmission blocking strategies against malaria if the required antibody titer can be obtained by immunization in human.

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PASSIVELY TRANSFERRED *P. FALCIPARUM* MSP1P42-SPECIFIC ANTIBODIES MEDIATE PROTECTION AGAINST CHALLENGE WITH BLOOD STAGES OF *PF*MSP1P19-TRANSGENIC *P. BERGHEI* PARASITES

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MSP1 is the major surface protein on merozoites and a prime candidate for a blood stage malaria vaccine. Preclinical and seroepidemiological studies implicate a role for anti-MSP1 antibodies in protection against malaria. These antibodies interfere with invasion or affect the growth of intra-erythrocytic parasites *in vitro*, depending on parasite strain. However, the biological activity of MSP1-specific antibodies is not fully captured by *in vitro* growth or invasion inhibition assays (GIA), which are frequently used to predict vaccine efficacy. GIA fail to address the potential role of cellular receptors that interact with antibodies and mediate anti-parasite activity through diverse antibody-dependent cellular mechanisms. Currently, this potentially cell-mediated functional activity of MSP1-specific antibodies can only be determined *in vivo*. Thus, we employed a PfMSP1p19-transgenic *P.berghei* parasite to test the ability of MSP1-specific antibodies to control parasitemia after challenge with infected erythrocytes. Various immune IgG preparations were tested in this model: a) IgG purified from rabbits immunized with MSP1p42 (FVO) using either complete Freund's adjuvant or an Adjuvant System, $AS01_8$ and b) Human IgG isolated from either high or low titer serum pools of malaria-naïve subjects immunized with MSP1p42 (FVO) adjuvanted with $AS01_8$. Purified IgG was injected intraperitoneally thrice (Day -1, 0, 1) and blood parasitemia was measured daily by qRT-PCR (Day 1-5) and by flow cytometry (Day 5-10). Lack of parasitemia was confirmed by qRT-PCR at the end of the study. Anti-MSP1p42- rabbit IgG conferred 40-50% sterile protection. Human anti-MSP1p42 IgG derived from the low titer pool protected40% of mice, while IgG derived from the high titer pool protected 80% of mice from developing parasitemia. These data suggest that the transgenic *P. berghei* mouse model could be useful in selection of candidate vaccines for future clinical studies.

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PARTICLE DELIVERY OF MALARIAL PROTEINS USING AN ATTENUATED STRAIN OF *SHIGELLA FLEXNERI*

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Particle-presentation of malarial antigens can significantly improve vaccine efficacy as show-cased by the RTS,S vaccine in which the circumsporozoite protein (CSP) is expressed as a fusion with hepatitis B surface antigen. While soluble CSP antigen plus adjuvant has not induced sterile protection, the RTS,S vaccine sterilely protects about 50% of malaria-naïve individuals. The advantages of particle delivery are that they can directly target antigen-presenting cells, contain immunostimulatory signals and provide increased epitope density thus assuring a potent immune stimulation. Our previous work using *E. coli* as particulate delivery platform has demonstrated that expression of malarial antigens at different cellular localizations (i.e., periplasmic space and outer membrane) modulates the type of immune response and can induce sterile protection against sporozoite challenge in murine models. In the present study, we use *Shigella*, a gram-negative bacterium, for particle presentations of malaria antigens and in the process potentially develop a dual-disease vaccination approach. Two malarial antigens were expressed in different compartments of strain 15G, an attenuated strain of *Shigella flexneri* 2a, to evaluate their immune responses in mice. The cell-traversal protein for ookinetes and sporozoites (CelTOS) was fused with the maltose binding protein, targeting it to the periplasmic space and the CSP was fused with the peptidoglycan associated lipoprotein in the outer membrane. We will report the results of bacterial dose selection, immunogenicity (humoral and cellular responses) and the protective efficacy against sporozoite challenge with either *P. berghei* or *Pf*CSP transgenic *P. berghei* sporozoites.

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A NOVEL GLYCOLIPID ADJUVANT STRONGLY ENHANCING THE CELLULAR IMMUNOGENICITY OF ADENOVIRUS-BASED MALARIA VACCINES IS ATTRIBUTABLE TO ITS LOCALIZED BIO-DISTRIBUTION

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A key strategy to a successful vaccine against malaria is to identify and develop new adjuvants that can enhance T cell responses elicited by a malaria vaccine. α-galactocylceramide (α-GalCer), a glycolipid that has been extensively investigated, is known to display a significant biological activity, including an adjuvant effect, by binding CD1d molecules and stimulating invariant NKT (*i*NKT) cells. Recently, we identified a novel synthetic α-GalCer analog, 7DW8-5, which can display a stronger adjuvant effect on the immunogenicity and efficacy of malaria vaccines in mice. Most recently, we have co-injected increasing doses of 7DW8-5 intramuscularly (i.m.) to rhesus macaques with an AdPfCA vaccine that consists of two Ad5-based vaccines each expressing the CS or AMA-1 antigen of *Plasmodium falciparum*, and found that 7DW8-5 could significantly enhance the level of malaria antigen-specific T cell responses without showing a significant side effect. Very surprisingly, we discovered that upon i.m. injection, α-GalCer, but not 7DW8-5, induced a systemic

production of cytokines including IFN-γ and IL-12 in the sera, whereas both glycolipids induced a similar level of systemic cytokine production upon their intravenous (i.v.) administration. Using labeled glycolipids with fluorophores, we found that the two glycolipids exhibited a distinctly different bio-distribution upon i.m. but not i.v. administration, resulting in only 7DW8-5 got trapped by DCs residing in the draining lymph nodes. The localized 7DW8-5 seems to facilitate the activation and maturation of lymph node DCs, thus improving the capability of DCs to prime malaria antigen-specific T cells and ultimately leading to its super adjuvant activity. Taken together, our study demonstrates a uniquely localized bio-distribution of our novel *i*NKT-activating glycolipid, 7DW8-5, upon its i.m. injection, which could lead to a potent adjuvant effect on the cellular immunogenicity of an adenovirus-based malaria vaccine not only in rodents but also in non-human primates.

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THE ROLE OF THE PROTEIN KINASE C SUPERFAMILY IN THE INNATE IMMUNE RESPONSE OF ANOPHELINE MOSQUITOES

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Anopheline mosquitoes are the primary vectors of medically important parasites in the genus *Plasmodium,* the causative agents of malaria. Malaria parasites undergo a series of complicated developmental transformations upon ingestion by *Anopheles* mosquitoes and during this process innate immune defenses can reduce parasite numbers significantly. While some mosquito anti-parasite effectors have been well characterized, the regulatory factors that control the timing and magnitude of these responses are poorly understood. The protein kinase C (PKC) superfamily consists of serine/threonine kinases that serve as central signaling molecules and regulators of a broad spectrum of cellular processes including growth, reproduction, and immunity. PKCs are highly conserved, ranging from seven isoforms in *Drosophila* to 16 isoforms in mammals, yet none have been identified in mosquitoes. Additionally, PKC-dependent signaling is central to the regulation of mammalian immunity and has been targeted aggressively for drug development. Despite conservation of the PKC superfamily and their potential as targets for transmission-blocking strategies for malaria, no direct connections between PKCs and the mosquito immune response exist. Here, we present the identification and characterization of six PKC superfamily members – PKCβ, PKCδ, PKCε, PKCζ, PKD, PKN – in *Anopheles gambiae* and *Anopheles stephensi*. Phylogenetic analysis of the anopheline PKCs confirmed subfamily assignments. All six PKCs are expressed in the midguts of *A. gambiae* and *A. stephensi*, indicating availability for signaling in a tissue that is critical for malaria parasite development. Inhibition of PKC enzymatic activity *in vitro* decreased NF-κB-regulated anti-microbial peptide expression in response to bacterial and parasitic specific factors. Further, PKC inhibition significantly decreased development of *P. falciparum* oocysts in *A. stephensi*, suggesting that PKC-dependent signaling is a positive regulator of the mosquito immune response and a potential target for transmission-blocking strategies.

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Anopheline mosquitoes are the primary vectors of medically important parasites in the genus *Plasmodium,* the causative agents of malaria. Malaria parasites undergo a series of complicated developmental transformations upon ingestion by *Anopheles* mosquitoes and during this process innate immune defenses can reduce parasite numbers significantly. While some mosquito anti-parasite effectors have been well characterized, the regulatory factors that control the timing and magnitude of these responses are poorly understood. The protein kinase C (PKC) superfamily consists of serine/threonine kinases that serve as central signaling molecules and regulators of a broad spectrum of cellular processes including growth, reproduction, and immunity. PKCs are highly conserved, ranging from seven isoforms in *Drosophila* to 16 isoforms in mammals, yet none have been identified in mosquitoes. Additionally, PKC-dependent signaling is central to the regulation of mammalian immunity and has been targeted aggressively for drug development. Despite conservation of the PKC superfamily and their potential as targets for transmission-blocking strategies for malaria, no direct connections between PKCs and the mosquito immune response exist. Here, we present the identification and characterization of six PKC superfamily members – PKCβ, PKCδ, PKCε, PKCζ, PKD, PKN – in *Anopheles gambiae* and *Anopheles stephensi*. Phylogenetic analysis of the anopheline PKCs confirmed subfamily assignments. All six PKCs are expressed in the midguts of *A. gambiae* and *A. stephensi*, indicating availability for signaling in a tissue that is critical for malaria parasite development. Inhibition of PKC enzymatic activity *in vitro* decreased NF-κB-regulated anti-microbial peptide expression in response to bacterial and parasitic specific factors. Further, PKC inhibition significantly decreased development of *P. falciparum* oocysts in *A. stephensi*, suggesting that PKC-dependent signaling is a positive regulator of the mosquito immune response and a potential target for transmission-blocking strategies.

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ANTI-ADHESION MOLECULES INHIBIT *PLASMODIUM* INFECTION IN *ANOPHELES* MOSQUITOES

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Plasmodium gametocytes ingested by mosquitoes with blood meal undergo gametocytogenesis and fertilization in mosquito midgut and develop to ookinetes. Ookinetes penetrate through mosquito gut wall to reach the gut epithelia where they attach themselves to the underlying basal lamina and develop to oocysts. Oocysts mature, develop thousands of sporozoites, and eventually rupture and release sporozoites into the mosquito haemolymph. Sporozoites then invade mosquito salivary glands. All these steps are governed by a series of adhesion phenomena made possible by interaction between receptors on the parasites and ligands expressed on mosquito tissues. Sporozoite invasion of salivary glands is also controlled by receptor-ligand interaction. Here we investigated the effect of several disintegrins and a c-lectin, which are proteins that interfere with adhesion phenomena mediated by integrins, on the *P. berghei* development in *Anopheles stephensi.* After mosquitoes were infected with *P. berghei*, they were fed daily with either 1 ug/ml of seven different disintegrins, c-lectin, or sugar (control). The mosquitoes were then examined for oocyst infection at Day 11 post infection and for sporozoite infection at Day 16. Mosquitoes treated with echistatin and VP12 showed decreased numbers of oocysts averaging 20/msq as opposed to averaged 50/msq in the controls. Only 30-40 % of mosquitoes treated with echistatin or VP12 showed at least 10 sporozoites in their salivary glands, while 90 % of mosquitoes fed on sugar (control) did. The results show that these disintegrins interfered with the adhesion phenomena leading to a decrease in oocyst attachment to mosquito midgut and sporozoite invasion of salivary glands.

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SELECTION FOR CHLOROQUINE-SENSITIVE *PLASMODIUM FALCIPARUM* BY *ANOPHELES ARABIENSIS* IN SOUTHERN ZAMBIA

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The emergence of *Plasmodium falciparum* drug resistance poses a major obstacle for malaria control and elimination. Public health strategies are needed to delay or minimize escalation. Field observations point to a link between mosquito control and the prevalence of *P. falciparum* drug resistance, the origin of which has remained unclear. Here we show field evidence for natural selection of wild type chloroquine-sensitive malaria parasites by *An. arabiensis* in southern Zambia. We screened 753 *An. arabiensis* by PCR, of which 8% and 10% were positive for salivary gland and mid-gut *P. falciparum* infections, respectively. We typed *P. falciparum* in humans and mosquitoes at the chloroquine resistance conferring amino acid codon 76 of the *Pf*CRT gene. Our data showed that despite being acquired from humans within a few weeks, *P. falciparum* infections in mosquitoes were up to 10X more likely to bear wild type *Pf*CRT K76 than in humans (OR [95%CI]: 10 [4.3 - 25.3], $p < 0.001$, n = 370). We concluded that a sporogonic selection occurs against mutated *Pf*CRT 76T-bearing *P. falciparum* in mosquitoes, presumably owing to altered biological fitness. This strong selection would seem to explain the association seen in the field between mosquito control and prevalence of drug resistance. We hypothesize that through this sporogonic selection, mosquitoes contribute to restoration of chloroquine-sensitive K76 parasites after suspension of drug use in humans. Understanding the nature and direction of the sporogonic selection could be instrumental in rational curtailment of drug resistance in integrated malaria control or elimination programmes.

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DEVELOPMENT OF A NEW BIOMARKER OF EXPOSURE TO *ANOPHELES* BITES BASED ON HUMAN ANTIBODY RESPONSES TO SALIVARY PROTEINS: FROM THE CONCEPT TO THE APPLICATIONS

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The study of human-vector immune relationships could allow several applications for the control of vector-borne diseases. Indeed, some salivary proteins from blood-feeding arthropods could induced a specific immune responses in human populations exposed to arthropod vectors bites. One hypothesis is that human immune response and especially antibody (Ab) response to whole saliva of mosquito could be an epidemiological biomarker of human exposure to vector bites. In the objective to increase the specificity to vector exposure, the second step was to identify salivary proteins i) specific to *Anopheles* genus and ii) antigenic in individuals exposed to malaria. First, the identification of antigenic salivary proteins of mosquito by an immuno-proteomic approach was assessed. The second step was to design peptide sequences, from one selected mosquito salivary protein using a bioinformatic approach, taking into consideration i) their potential antigenic properties and ii) the absence of cross-reactivity with other arthropods/organisms. For malaria, the specific IgG Ab levels were then evaluated in African children in different context of malaria. From five peptides, only one peptide (gSG6-P1) presented all criteria to be an optimal candidate biomarker for evaluating human exposure to An. gambiae and An. funestus bites and interestingly for evaluating the efficacy of vector control. This new "salivary" biomarker of *Anopheles* exposure could be used as a geographic indicator for mapping the risk of malaria transmission and especially in low *Anopheles* density conditions, where entomological methods are limited in sensitivity (dry season, altitude or urban malaria). It also represents a direct criterion of efficacy in the evaluation of anti-vector strategies.

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MALARIA IN SCHOOL CHILDREN UNDER A NEW POLICY OF UNIVERSAL COVERAGE OF NETS: RECENT DATA FROM MALI AND SENEGAL

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Malaria control has traditionally focused on pregnant women and children under five years, in whom the risk of malaria-related mortality is greatest. Yet studies have shown that older school-age children can also benefit from malaria control, with potential gains for both health and education. Insecticide-treated nets are a cornerstone of malaria prevention efforts, but in many countries net usage is lowest in school-age children compared with younger children and adults. Universal coverage of nets will help address this gap, and is increasingly being adopted into policy by national control programmes in malaria-endemic countries. Senegal and Mali recently introduced universal coverage of nets, with national roll-out of community-wide distributions of long-lasting insecticidal nets (LLINs) starting in 2010 and 2011 respectively. The coverage of LLINs amongst schoolchildren in these two countries was examined through school surveys 6-12 months after the net distributions, and the prevalence of malaria parasitaemia and anaemia measured at the end of the transmission season. Data was collected in 38 primary schools (1900 children) in Sikasso, Mali and 6 primary schools (865 children) in Kedougou, Senegal. Our data provide evidence that the new strategy was successful in achieving coverage in this previously neglected age group: reported and observed use of nets was high in both countries, with over 80% of schoolchildren (age 7-14 years) using nets. Yet paradoxically, levels of malaria infection remained high. Overall 83% of primary schoolchildren in Mali (range: 46-98%), and 54% of schoolchildren in Senegal (range: 20-81%) had asymptomatic parasitaemia in December 2011. Factors which may account for this apparent paradox will be discussed, and data presented on patterns of net use by child and household characteristics, including time of going to bed and discontinued use of nets in later months. We shall also present findings from an alternative malaria control strategy in schools, intermittent parasite clearance, which is currently being trialed in these two sites.

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THE EFFECT OF LIMITED RESIDUAL LIFE OF INSECTICIDE AND OUTDOOR BITING ON MALARIA INFECTION IN CHILDREN ON BIOKO ISLAND, EQUATORIAL GUINEA: AN EXAMINATION OF TWO KEY ASSUMPTIONS OF INDOOR RESIDUAL SPRAYING

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Malaria is endemic on Bioko Island, Equatorial Guinea, with year round transmission. In 2004 an intensive malaria control strategy primarily based on Indoor Residual Spraying (IRS) was launched. The limited residual life of IRS poses particular challenges in a setting with year round transmission such as Bioko. Recent reports of outdoor biting by An. gambiae are a further cause for concern. In this study the effect of the short residual life of bendiocarb insecticide and of children spending time outdoors at night on malaria infection prevalence was examined. Data from the 2011 annual malaria indicator survey and from standard WHO cone bioassays were used to examine the relationship between time since IRS, mosquito mortality and prevalence of infection in children. Children spending time outside at night and the association of this behavior with malaria infection were also examined. Prevalence of malaria infection in 2 to 14 year-olds was 18.4%, 21.0% and 28.1% in communities with median time since IRS of three, four and five months respectively. After adjusting for confounders, each extra month since IRS corresponded to an odds ratio (OR) of 1.44 (95% CI 1.15 - 1.81) for infection prevalence in 2 to 14 year-olds. Mosquito mortality was 100%, 96%, 81% and 78%, at month two, three, four and five respectively after spraying. Only 4.1% of children spent time outside the night before the survey between the hours of 10pm and 6am and were not at a higher risk of infection (OR 0.87, 95% CI 0.50 - 1.54). Sleeping under a mosquito net provided additive protection (OR 0.68, 95% CI 0.54 - 0.86). The results demonstrate the epidemiological impact of reduced mosquito mortality with time since IRS. The study underscores that in settings of year round transmission there is a compelling need for longer lasting IRS insecticides, but that in the interim high coverage of long lasting insecticidal nets (LLINs) may ameliorate the protective effect conferred by current shorter lasting IRS insecticides.

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EARLY MORNING BITING BY *ANOPHELES* VECTORS: A POTENTIAL RISK PERIOD FOR MALARIA INFECTION IN AN AREA WITH HIGH AND SUSTAINED USE OF INSECTICIDE TREATED BED NETS IN WESTERN KENYA

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As malaria vector control activities using insecticide-based interventions such as indoor residual spraying and insecticide treated nets (ITNs) expand, the question emerges as to how these activities shape the distribution, population and behavior of the vectors; as these processes will influence the effectiveness of each intervention. Outdoor and early evening biting have been suggested as possible vector behavior changes resulting from indoor vector control interventions. Therefore, we conducted a study during the peak malaria season in an area of high ITN ownership in western Kenya to quantify the biting behavior of the local malaria vectors, and related results to the behavior of people in the community. A total of 150 adult men were recruited as human landing catchers who collected and stored mosquitoes hourly at indoor and outdoor fixed positions, from 5 PM to 7 AM, for 4 nights per week, in a total of 75 villages for a period of 6 weeks. The main malaria vectors were *Anopheles* arabiensis (n=153, sporozoite rate SR=0.04); *A. gambiae* s.s (n=241, SR=0.12) and A. funestus (n=1169; SR=0.09). More than a third of bites by each of the main vectors occurred outdoors. However, by 9 PM, 88% of the human population was indoors and were presumably not at risk for malaria infection by outdoor biting mosquitoes. Indoors, the peak biting for all three species occurred after midnight, and biting continued to 7 AM. Net use was high with 77% of the population reporting the use of a net the previous night. By 11 PM, 96% of the population reported going to bed and those who reported using a net were likely at a low risk of mosquito bites and malaria infection. In the morning hours, about 52% of the population was awake before 6 AM, a time when vector mosquitoes, particularly An. funestus were still active, suggesting a window of risk for malaria infection. The temporal distribution of risk of infectious bites among the population and implications for vector control will be discussed.

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CLUSTER-RANDOMIZED TRIAL OF TEXT MESSAGE REMINDERS TO RETAIL STAFF OF APPROPRIATE PRACTICES FOR DISPENSING ARTEMETHER-LUMEFANTRINE IN DRUG SHOPS IN TANZANIA: EFFECT ON DISPENSER KNOWLEDGE AND PATIENT ADHERENCE

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Patient adherence, the extent to which patients promptly and correctly take the full course of a drug, is a key component in ensuring drug effectiveness. As artemisinin-based combination therapies (ACTs) for malaria become more widely available in the private sector, there are concerns that patient adherence might be low due to insufficient or incorrect advice provided by dispensers with limited training. In this cluster-randomized trial in drug shops in southern Tanzania, we assess the effect of text message reminders to retail staff on advice to provide when dispensing artemether-lumefantrine (AL) on dispenser knowledge and patient adherence. Of 72 randomly selected drug shops in Mtwara region, 36 were randomized for dispensers to receive text message reminders once per day five days per week beginning a month prior to the study. No intervention was delivered in the control arm. Patients desiring to purchase ACT at study drug stores were eligible to receive AL from a mixed supply of regular blister packs and identical-looking blister packs containing devices to record the date and time each blister was opened to remove pills. From each arm, 468 patients receiving study AL were followed up at home a minimum of 75 hours after drug purchase; consenting patients or their caregivers were administered a detailed questionnaire about when and how each dose of AL was taken. Patients were asked to present their blister packs for a pill count and extraction of timestamp data. Following patient data collection, dispensers were interviewed regarding their knowledge of AL dispensing practices, and mobile phone usage and receipt of malaria-related messages. Using data from questionnaires, pill-counts, and timestamps, we will report the effects of the intervention on dispensers' knowledge, the proportion of patients completing all doses within 75 hours of purchase and those adhering to the correct timing of each dose, and the advice patients received from the dispenser. These data will be useful for designing strategies to enhance the effectiveness of ACTs in the private sector.

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ANALYSIS OF FACILITY-LEVEL STOCKOUTS OF ACTS IN ZAMBIA: THE IMPACT OF INVENTORY MANAGEMENT

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Despite remarkable and successful recent improvements efforts by the government and its partners, the current public distribution system of essential medical drugs in Zambia still results in low availability to patients relative to private sector standards. Many possible causes have been cited, including procurement financing and processes, supply capacity, communication and road infrastructure, distribution resources and planning methods, personnel staffing and training, coordination among stakeholders. A field experiment in Zambia's public distribution system conducted from Q3 2009 to Q2 2010 involved a high adherence to recommended inventory control policies and offers an opportunity to isolate their impact. To do so we collected daily clinic storeroom stock levels of Arthemeter-Lumefantrin (AL) antimalarial products in up to 90 facilities through photography and manual transcription, then used that data to estimate demand patterns and service levels. Delivery lead-times and estimates of monthly facility accessibility were obtained through survey of health workers. Monthly national warehouse stock levels were extracted from a software database. A simulation model was constructed to reproduce and interpret observations of stock-out patterns. We found that up to 30% of surveyed facilities stocked out of all AL products at certain times of the year despite ample inventory being available at the national warehouse. The simulation model closely reproduced these results and linked them to the use of average past monthly issues and failure to capture lead-time variability in current inventory control policies. These results suggest that inventory control policies widely recommended and used for distributing medicines in Sub-Saharan Africa directly account for some of the stockouts observed in situations involving demand seasonality and/or clinic access interruptions. They also suggest specific improvement opportunities for pharmaceutical inventory control systems that include digital transmission of inventory transactions through mobile wireless devices, standard forecasting algorithms and mathematical optimization.

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A REVIEW OF THE CAUSES OF ACT STOCK-OUTS IN BURUNDI

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Prompt treatment of malaria cases with an effective antimalarial is a key global strategy for malaria control. Despite global efforts to scale up the use of artemisinin-based combination therapies (ACTs), coverage across Africa remains poor, with public sector health facilities frequently plagued by stock-outs. The causes of stock-outs vary, but often reflect poor planning and weak supply chain management systems. Data from public health facilities in 6 African countries illustrated that in some cases up to 90% of health facilities lacked the full range of weight-specific packs of the recommended ACT treatments in stock. Stock-outs often last several weeks, leaving malaria patients dangerously vulnerable. We analyzed the root causes of these stock-outs in Burundi using both record reviews and in-depth interviews with providers at the national, district, and facility levels between June and December 2011. Results indicated that districts required five signatures with their monthly requisition for ACTs, which led to delays and a resignation to use other treatment options or split blister packs of other age groups, which skewed consumption data. The districts did not receive sufficient stock to cover their health facilities, and little provision was made for safety stock or emergencies, resulting in partially filled orders. As soon as districts receive monthly orders, they repeat the process without allowing time to monitor stock sent to the facilities. The time between preparing an order and distribution to the facilities is long, and emergency procurements are frequent and expensive. The formulas used to forecast needs at the multiple levels are inconsistent; in addition, data are sometimes "created" in reports to place an order and to meet performance targets. Staff also appeared to be complacent regarding the effect of stock-outs on patient outcomes. While interventions to avert some challenges are being implemented, more efforts are needed to ensure uninterrupted availability of ACTs and to promote the importance of these efforts at all levels.

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QUALITY OF UNCOMPLICATED MALARIA CASE MANAGEMENT IN MALAWI-FINDINGS FROM A NATIONAL HEALTH FACILITY SURVEY

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Quality malaria case management is dependent on patients being appropriately assessed, diagnosed, and treated with artemesinin-based combination therapy (ACT) for uncomplicated malaria. We conducted a nationally representative cross-sectional health facility survey in Malawi to examine malaria case management quality and assess factors related to correct treatment. We sampled 107 public health centers and hospitals in all 29 districts of Malawi in April-May 2011, during peak malaria transmission. In all, 2,019 patients seeking curative care at outpatient departments were interviewed after their consultation, and blood smears were taken. Malaria was defined as fever or history of fever and malaria parasitemia on exit interview blood smear. Logistic regression was used to examine factors associated with correct treatment, defined as ACT prescription for patients with malaria. Thirty-four percent of all patients presenting to facilities in Malawi had malaria, including 46% of children <5 years and 27% of patients ≥5 years ($p<0.001$). Among patients with malaria, 67% received correct treatment; the most common reason for incorrect treatment was missed diagnosis (27%). Clinicians did not assess fever/history of fever in 27% of all patients. Only 21% of patients were tested for malaria using microscopy, and rapid diagnostic tests were not yet available. Overtreatment was common with 31% of patients without malaria prescribed an ACT. Patient-level factors, including high temperature (adjusted odds ratio (aOR) = 3.3; 95% confidence interval (CI) 3.3-5.5), spontaneous complaint of fever (aOR = 4.0; 95% CI 3.3-7.2), and complaint of cough (aOR = 0.3; 95% CI 0.2-0.5) were significantly associated with correct treatment. Health worker- or facility-level factors were not. Malawi has a high burden of uncomplicated malaria, but both failure to deliver correct treatment and overtreatment are common. Improved assessment of fever and increased parasitological confirmation of malaria diagnosis are critical to improve malaria case management.

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ADVERSE DRUG EVENTS RESULTING FROM USE OF DRUGS WITH SULFONAMIDE AND ARTEMISININ-BASED ANTIMALARIALS: FINDINGS ON INCIDENCE AND HOUSEHOLD COSTS FROM THREE DISTRICTS WITH ROUTINE DEMOGRAPHIC SURVEILLANCE SYSTEMS IN RURAL TANZANIA

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Antimalarial regimens including sulfonamide and artemisinin derivatives have been deployed in many parts of the world in an effort to halt the acceleration of antimalarial drug resistance problem. Access to these drugs has faced multiple obstacles including availability, acceptability, and adherence. Meanwhile, weak public health infrastructures and drug regulatory authorities prevalent in most malaria endemic countries, particularly in sub-Saharan Africa, are partly responsible for poor post-marketing surveillance and have enhanced the proliferation of fake antimalarials. We used active and passive surveillance to identify and document antimalarial-associated adverse drug reactions (ADR) in three rural districts of Tanzania with high malaria transmission. Clinicians were trained to identify, categorize and report ADR cases linked to sulfadoxine/pyrimethamine (SP) and artemisinin (AS) use. Additional questions relating to demographics, care-seeking and treatment costs were asked. A total of 95 suspected ADR cases were identified. 79 were traced and successfully classified. 67 (85%) of the 79 cases were related to use of SP and/or AS antimalarial drugs. 51% of the 67 cases were classified as 'probable' and 49% were classified as 'possible' ADR events. Annual ADR incidence per 100,000 was calculated at 5.6 for AS/SP and 25.0 for SP monotherapy. Treatment costs per episode ranged from a median of US $2.00 for those making a single visit to US $21.13 for patients with 4 visits to healthcare providers. Drug costs constituted 43% of the treatment costs. Faith-based and NGO facilities were the most expensive source of care. 85% of the patients used out-of-pocket funds to pay their bills. 21% of the patients had to sell assets or borrow from relatives to settle their bills. Costs of treatment of ADR episodes were substantially catastrophic.

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

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

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N-ACETYL TRANSFERASE GENE TYPE 2: PREDOMINANCE OF SLOW ACETYLATORS AND EFFECT ON RESPONSE TO ARTESUNATE AMODIAQUINE

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Inter individual differences in the metabolism of the antimalarials could be due to polymorphism of NAT2 gene. We determined the genotypic frequencies of single nucleotide polymorphism (SNP) of NAT2 gene and it's implication in antimalarial treatment during a vitamin A and zinc supplementation intervention in children less than 5 years in Bangolan, Cameroon. A total of 100 children aged 6 to 24 months were recruited into the study after obtaining informed consent from parents or guardians. Participants were randomized to receive vitamin A +placebo or vitamin A+Zinc supplements. All participants received artesunate-amodiaquine(ASAQ) -toddler 50/135mg at baseline to clear any parasites, vitamin A administered and followed up for 30days. This was followed by daily administration of Zinc or placebo and follow up for 6 months for incidence of clinical malaria and other diseases. Blood was spotted on filter paper for DNA extraction by chelex method. RFLP-PCR was performed with restriction enzymes KpnI, TaqI, and BamHI for detection of NAT2*5, NAT2*6, NAT2*7 SNPs respectively. Allelic frequencies and phenotypes were compared between participants with or without adverse reactions . A total of 55% of the participants had slow acetylator, 30% intermediate acetylator, 11% rapid acetylator and 4% an unknown genotype. NAT2 genotypes observed to be associated with susceptibility to develop anorexia were NAT2*5/5 (OR=13,000) and NAT2*4/6 (OR=6,538). Those likely to develop fever were NAT2*4/7 (OR=5,082), NAT2*5/6 (OR=2,389), NAT2*6/7 (OR=1,481) and NAT2*5/7 (OR=1,156). Those likely to develop fever of unknown etiology were NAT2*6/6 (OR=23,467), NAT2*4/5 (OR=2,933), NAT2*5/5 (OR=2,048) and NAT2*4/6 (OR=1,026). Those likely to develop skin rash were NAT2*4/5 (OR=2,857), NAT2*5/7 (OR=2,483), NAT2*6/7 (OR=1,385), and NAT2*4/7 (OR=1,357). Those likely to develop cough, catarrh (common cold) and fever were NAT2*4/6 (OR=2,255), NAT2*6/6 (OR=1,895), NAT2*4/5 (OR=1,850), NAT2*5/5 (OR=1,200) and NAT2*5/5 (OR=1,016). The slow acetylator genotype NAT2 gene was the most predominant in the study population. Both slow and intermediate acetylators were more likely to the develop adverse reactions to ASAQ, vitamin A and Zinc supplements.

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EXPLORING HOW LARGE-SCALE IMPLEMENTATION OF MALARIA CONTROL PROGRAMS MEDIATES THE RELATIONSHIP BETWEEN HOUSEHOLD SOCIOECONOMIC STATUS AND VARIOUS CHILDHOOD MALARIA CONTROL INDICATORS: EXPERIENCE FROM THREE PRESIDENT'S MALARIA INITIATIVE COUNTRIES IN SUB-SAHARAN AFRICA

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Following the first Global Malaria Eradication Program in the 1950's malaria was confined almost exclusively in the poorest nations of the world, particularly in sub-Saharan Africa and Southeast Asia. While macroeconomic studies consistently show a strong and stable relationship between malaria and poverty, most microeconomic studies have largely been inconclusive. This study explores how variations in implementation of large scale malaria control programs may explain the reason why microeconomic studies remain inconclusive. It sets out to explain the critical role played by large scale implementation of malaria control strategies such as those financed by Global Fund and the President's Malaria Initiative (PMI) in mediating the relationship between households' socioeconomic status (wealth, education and place of domicile) and key malaria control indicators. The study focuses on malaria parasitemia, bed-net ownership and bed-net use among children aged less than five years as key outcome variables of interest. We analyzed Malaria Indicator Survey data for the first three PMI countries: Angola, Tanzania and Uganda. A multilevel-hierarchical cluster analysis restricted to malaria program implementation regions was used. SES interaction terms with malaria program implementation were found to have significant bearing across the three countries. In Angola, programs were more likely to benefit households headed by individuals or mothers with higher education levels whereas household wealth status was less important. In Tanzania, wealth, education and living in urban or rural settings were all significant determinants of which households benefited more from the programs while in Uganda programs were more likely to benefit poorer households. Following these findings, policy relevant conclusions are drawn to help design more pro-poor malaria control policies in light of the renaissance of malaria eradiation policy debates.

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PREVENTION OF NEONATAL HYPOTHERMIA IN SOUTHERN PROVINCE, ZAMBIA

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Newborn hypothermia is associated with increased neonatal mortality. Zambian guidelines recommend facility-based delivery by skilled birth attendants and immediate postpartum skin-to-skin care to provide thermoprotection of the neonate. This study assessed institutional capacity to prevent neonatal hypothermia in Zambia. We conducted comprehensive health center (HC) surveys in Southern Province, Zambia, and pregnant women were recruited at the same HCs during routine antenatal care to participate in a neonatal study (ZamCAT). Enrollees were interviewed 4 days post-delivery about the delivery and immediate postpartum care. Of the 90 primary HCs surveyed, only 8.8% had a neonatal warmer and 6.7% had heat control for the delivery room. When HC directors were asked about delivery practices, 36.7% said the newborn was placed the mother's abdomen after delivery, 46.7% put the baby next to the mom and 15.6% placed the baby in a cot. Nearly all HCs (94.4%) reported drying and wrapping the baby in a new cloth, and, in the last month, 92.2% recommended skin-to-skin contact to new mothers. Among 9,816 deliveries [63% at a facility, 36% at home], the baby was placed on mother's skin after delivery 49.9% of the time; this was significantly higher in facility compared to home deliveries ($p<0.001$). Women delivered by a nurse/midwife or trained TBA were more likely to have the baby put on the mother's skin afterwards compared to those delivered by family members, self or untrained TBAs (61.8% vs. 21.5%, $p<0.001$). In 98% of deliveries, the baby was wrapped in a dry cloth; this did not differ by delivery location. Southern Province health centers are not well equipped to prevent neonatal hypothermia although evaluation of actual practices suggests that efforts are made to warm the newborn and recommend skin-to-skin care. These practices are less common in home deliveries, thus increasing risk of hypothermia in newborns delivered by unskilled birth attendants.

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USING CURRENT AND EXTENDED ROUTINE PREVENTION VISITS TO HEALTH FACILITIES ACHIEVE HIGHER COVERAGE WITH CHILD-SURVIVAL INTERVENTIONS IN SUB-SAHARAN AFRICA

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Interventions to improve child survival in sub-Saharan Africa are frequently administered during mass campaigns (MCs), particularly for interventions targeting children 1-4 years old. Integrated delivery of interventions in MCs, including Vitamin A supplementation, insecticide-treated bednets, supplemental immunizations, and deworming, has been promoted to decrease program costs and to expand coverage. However, integrated preventive services have long been provided during routine preventive visits (RPVs) to health facilities by pregnant women and mothers and their children during the child's first year of life. While the interventions offered during RPVs are currently limited, they could be expanded and offered as well at new RPVs scheduled up to the age of five. To assess RPVs as a platform for expanded service delivery and compare them to MCs, we analyzed data from Demographic and Health Surveys in 12 sub-Saharan African countries in which mothers were asked about receipt of services for themselves or their children in one or more MCs (median number of MCs: 4.5; range: 2-11). RPV coverage demonstrating access (the percentage of mothers seen at least once for an RPV) was high in all countries (range: 80.6 [Nigeria]-99.9% [Swaziland]; median: 96.7), typically exceeding the percentage of eligible 1-4 year-old children receiving an intervention in at least one MC (range: 36.3 [Sierra Leone]-89.5% [Eritrea], median difference: 28.1 percentage points). The median number of RPVs among mothers of 1-4 year-old children ranged from 4.5 in Niger, to 12.9 in Swaziland. The percentage of children aged 1-4 years missed by all MCs but whose mothers and made at least one RPV ranged from 62.7% in Nigeria to 99.5% in Sao Tome & Principe. The median number of RPVs among these children ranged from 2.3 in Niger to 11.0 in Ghana. Among 1-4 year-olds whose mother made no RPV, the percentage receiving at least one MC intervention for which they were eligible was lower (range: 0.0 [Sao Tome & Principe]-35.5% [Niger]; median 4.8%). Current and extended RPVs may reach children missed by MCs, and are potentially an effective alternative to MCs for delivering some child-survival interventions, particularly to 1-4 year-old children.

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IMPACT OF A BRIEF IN-HOME NEONATAL HEALTH PROMOTION ON SELF-REPORTED BIRTH AND NEONATAL CARE PRACTICES AMONG PRIMIPAROUS WOMEN IN THE THIRD TRIMESTER IN RURAL BANGLADESH

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Over 75% of neonatal deaths occur in the first week of life. Community-based promotion programs that have promoted safe birthing and early infant care practices have decreased neonatal mortality; but they included prolonged staff training and antenatal visits as early as 12-16 weeks gestation. Although desirable, reaching women early in pregnancy with highly trained staff is difficult and expensive, potentially limiting the scale of these programs. We sought to describe the behavioral impact of safe birthing and neonatal care messages delivered to primiparous women in the third trimester as part of a randomized controlled trial of handwashing promotion in rural Bangladesh. We promoted delivery at a medical facility, use of a clean delivery kit for a home delivery, recognition of maternal and neonatal danger signs, and essential neonatal care to all participants and their families. Field workers received four days of training and delivered the messages during one home visit between 33 and 35 weeks gestation and two visits within one week after birth. We compared self-reported changes in knowledge and beliefs of these practices before and after the intervention. Of 250 women in the study, 212 completed interviews before and after the intervention. Prior to the intervention, 57% of the women had ≥1 prenatal visit to a health care provider, which increased to 94% after the intervention ($p<0.01$). Only 2% planned to deliver at a medical facility but 41% reported delivering at a medical facility ($p<0.01$). Before the intervention, 32% of women reported a foreign substance (such as oil or dung) should be placed on the umbilical cord after cutting, and 72% agreed a baby should be bathed immediately after birth. However, only 6% reported placing anything on the cord after it was cut ($p<0.01$) and 2% of neonates were reportedly bathed <5 hours after birth ($p<0.01$). This program requiring minimal training of field staff resulted in reports of improved birth and neonatal care practices compared to reported prior beliefs. Neonatal care and birthing practices can be improved, even when women are identified late in pregnancy. A brief training of community health workers may be feasible and effective for reducing risky health behaviors in the antenatal and neonatal period, and may be scalable.

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THE ASSOCIATION BETWEEN COGNITION AND ACADEMIC ACHIEVEMENT IN UGANDA CHILDREN SURVIVING MALARIA WITH NEUROLOGICAL INVOLVEMENT

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An understanding of the contribution of different cognitive abilities to academic achievement in children surviving cerebral injury can guide the choice of interventions to improve cognitive and academic outcomes. This study's objective was to identify which cognitive abilities are associated with academic achievement in children after an episode of malaria with neurological involvement (MNI). 62 children with a history of MNI were assessed for cognitive ability (working memory, reasoning, learning, visual spatial skills, attention) and academic achievement (arithmetic, spelling, reading) three months after recovery from the illness. Linear regressions were run for each academic score with the five cognitive outcomes entered as predictors. Adjusters entered in the analysis were age, sex, education level, nutritional status and quality of the home environment. Exploratory factor analysis (EFA) and structural equation models (SEM) were used to determine the nature of the association between cognition and academic achievement. In regression of a single academic score on all five cognitive outcomes and adjusters, only Working Memory was associated with Reading (coefficient estimate=0.36, 95% confidence interval=0.10 to 0.63, $p<0.01$) and Spelling (0.46, 0.13 to 0.78, $p<0.01$), Visual Spatial Skill was associated with Arithmetic (0.15, 0.03 to 0.26, $p<0.05$), and Learning was associated with Reading (0.06, 0.00 to 0.11, $p<0.05$). A single latent cognitive factor was identified using EFA. The SEM demonstrated a strong association between this latent cognitive ability and each academic achievement measure ($P < 0.0001$). No additional association between the academic scores and the individual cognitive measures was found beyond the latent cognitive ability. Academic achievement is best predicted by a latent variable, cognitive ability, which captures most of the variation in the individual cognitive ability measures. EFA and SEM can help to define how cognitive testing outcomes relate to academic achievement in children with disease-associated brain injury.

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BELIEFS AND CULTURAL PRACTICES TOWARDS MEASLES AND MEASLES VACCINATION PROGRAMS IN A MULTI-ETHNIC URBAN NEIGHBORHOOD IN KENYA: A QUALITATIVE STUDY

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A recent measles outbreak in Kenya began in late 2010 and by April 2011 had spread across the country, with the highest number of cases reported in Eastleigh, Nairobi, a community with a high proportion of refugees and migrants from neighbouring countries. To better understand cultural perspectives and community awareness of measles, and assess response to immunization activities, we conducted a series of focus group discussions (FGDs) in Eastleigh. Six FGDs were held (during April 23-29, 2011) before a supplementary immunization activity (SIA) for children <5 years and another 6 FGDs were held (during May 18-20, 2011) after the SIA. Between 6 and 10 individuals matched for primary language and gender participated in every session. Sessions were facilitated by persons with similar primary language and gender using facilitation guides with similar questions for all the groups. The sessions were recorded, transcribed and translated into English. Qualitative data were analyzed using NVivo 2.0. A total of 103 individuals (mean age, 30.5 years) representing three language groups (Oromo, Somali and Swahili) participated in the 12 discussions. Participants in all groups were able to identify measles and associated it with poverty, poor sanitation and dirty environment. The Oromo and Somali speakers mentioned home remedies as first-line therapy. Cost, long queues, distance to immunization sites, perceived discrimination by non-nationals, lack of understanding of health messages due to language barriers, belief that injections could cause death or exposure to disease, and belief that vaccinated children were not protected were some of the barriers to vaccination mentioned. Somali and Oromo participants recommended providing information through trusted community leaders and community health workers who speak their primary languages. Failure to provide linguistically and culturally appropriate health education materials may negatively impact disease prevention efforts in this setting with ethnically diverse populations.

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PRACTICES OF ANTIBIOTIC USE IN CHILDREN LESS THAN FIVE OF MEDICAL PERSONNEL IN PRIMARY CARE CENTERS IN PERI-URBAN AREAS OF LIMA, PERU

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The increase of antibiotic resistant pathogens acquired in the community is a growing problem worldwide which requires prompt intervention. The overuse and misuse of antibiotics in children is a practice rooted in developing countries, and it has been assumed that the use of antibiotics without prescription is one of the main causes of this misuse. However, previous studies showed that physicians had prescribed more than 80% of the antibiotics used and have the main responsibility in the overuse of antibiotics. The objective of the study was to describe the practices of antibiotics use in children under 5 years by the medical staff of primary health care. A structured questionnaire was applied in 218 general practitioners of primary care facilities of three districts of peri-urban Lima. It consisted of 6 typical clinical cases that may occur in children less than 5 years. 75.6% of the doctors affirmed that of the total of patients attended, more than 25% were children under the age of 5 years. Only 3.2% doctors had received training in pediatric care. When asked if necessary the use of antibiotics in the case of common cold, 15.6 would used an antibiotic, mainly amoxicillin (76.5%).78.9% of the physicians would use antibiotic in dysentery, mainly furazolidone (39.9%) and TMP-SMX (43.9) . 84.2% of the doctors would recommend an antibiotic for pharingitis and would use amoxicillin (54.3%) and amoxicillin -clavulanic acid (22.3%). 33.2% of the doctors responded that an antibiotic was needed for watery diarrhea treatment, they mainly used furazolidone(42.3%) and TMP-SMX (40.8%). 73.3% would recommend an antibiotic for bronchospasm. 28.3% would use amoxicillin -clavulanic acid and 28.9% amoxicillin. 98.1% would recommend an antibiotic in the case of pneumonia, mainly amoxicillin -clavulanic acid (30.7%) and cephalosporins (26.7%). Approximately half of patients treated in the study primary care establishments are children under five. However the doctors didn't receive training in pediatric care. An overuse of prescribed antibiotics in children less than 5 years was observed, especially in diagnoses as watery diarrhea, pharyngitis and bronchospasm. Misuse of antibiotics that are not considered first line of action on the pathogens or to which the pathogens are highly resistant show that training of medical personnel should be improved in order to reduce unnecessary antibiotic use.

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RECONSTRUCTING THE POPULATION HISTORY OF *WUCHERERIA BANCROFTI* IN A POST-MDA REGION

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Wuchereria bancrofti (Wb) is the primary causative agent of lymphatic filariasis (LF). Our studies of LF in Papua New Guinea have shown that it is possible to reduce the prevalence of Wb in human and mosquitoes through mass drug administration (MDA; diethylcarbamazine with/without ivermectin). While MDAs through 1998 significantly reduced prevalence of Wb infection, interruption has allowed parasite populations to recovering to pre-MDA levels. We collected genetic data with the objectives to i) reconstruct Wb population dynamics post-MDA, ii) document contemporary levels of genetic diversity, and iii) differentiate mechanisms of population connectivity. We sequenced Wb infections from 17 patients across 8 villages encompassing both high and moderate annual transmission potentials (ATP).We confirmed the presence of a genetic bottleneck consistent with past MDA treatment with a successive period of exponential growth following treatment interruption. We characterized 175 unique maternal haplotypes currently segregating in the Wb population, with one common haplotype present in 75% of infections. Finally we describe the spread of haplotypes between villages corresponding to the period of population growth following interruption of MDA. We conclude that while the MDA was successful in reducing the Wb genetic diversity, it was not prolonged enough to eliminate all genetic diversity. Interruption of treatment allowed the parasite population to recover and consequently disperse across the landscape via host and vector migration. We hypothesize that through the combined use of long-lasting insecticide treated bed nets (LLINs) in conjunction with MDAs we can eliminate all but the most common haplotypes as well as prevent migration of drug resistant strains both among patients and among villages. Through examining genetic diversity, we have been able to make insights into the demographic history of the parasite population and estimate the most effective strategies to reduce genetic diversity.

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COMPARATIVE PROTEOMICS OF *WOLBACHIA* STRAINS BETWEEN INSECTS AND NEMATODES

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The symbiont *Wolbachia* is of intense interest for tropical medicine, both as a drug target in filarial nematodes and as an inhibitor of pathogen transmission in insect vectors. Research on *Wolbachia* has been accelerated by genome sequencing from the major taxonomic "supergroups", including "A" (strain *w*Mel from *Drosophila melanogaster*, 1.3 Mb), "C" (*w*Oo from *Onchocerca ochengi*, 1.0 Mb) and "D" (*w*Bm from *Brugia malayi*, 1.1 Mb). However, proteomic analysis of *Wolbachia* remains scant, despite its potential to illuminate the apparent divide between "parasitic" (group A) and "mutualistic" strains (C and D). Here, we present absolute abundance data for ~30% of the *w*Mel proteome, compared with semi-quantitative estimates for *w*Bm and *w*Oo. Strikingly, the chaperonin GroEL represents 20% of *w*Mel protein, and also dominates in *w*Bm and *w*Oo, alongside six other conserved proteins [*Wolbachia* surface protein, elongation factor (EF)-Tu, co-chaperonin GroES, chaperone DnaK, peptidoglycan-associated lipoprotein, and a porin]. Despite the larger genome of *w*Mel, only two proteins absent from *w*Bm and *w*Oo (a hypothetical protein and a NAD-dependent epimerase) are highly expressed in *w*Mel; although EF-G, heat-shock protein 90 and ribosomal protein L7/L12 are quantitatively elevated. Surprisingly, the profiles of proteins involved in the stress response, nucleotide salvage, transcription and DNA binding are more similar between *w*Bm and *w*Mel than *w*Bm and *w*Oo. The abundance of many proteins in *w*Bm and *w*Oo is not concordant, with increased representation of Zn peptidases, Lon protease and an ankyrin protein in *w*Bm; in contrast with ClpB protease, the copper chaperone SCO1, and oxoglutarate dehydrogenase in *w*Oo. However, shared overrepresentation of two proteins (ATP synthase and HtrA protease) may constitute a "mutualistic signature". Thus, proteome evolution in *Wolbachia* is shaped by compensatory mechanisms to maintain protein metabolism during genome reduction. However, that hypothesis that ATP is a key metabolite provisioned by the mutualistic strains is also supported.

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WOLBACHIA-LIKE TRANSCRIPTS AND PROTEINS IN THE WOLBACHIA-FREE FILARIAL PARASITE *ONCHOCERCA FLEXUOSA*

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Most filarial parasites that infect humans require *Wolbachia* endobacteria for normal development and reproduction. It would be interesting to know how *Wolbachia*-free filarial worms function without an endosymbiont. We have previously reported that two *Wolbachia*-free filarial species contain *Wolbachia*-like sequences in their nuclear genomes and that some of these sequences are expressed at the RNA level in a tissue- and stage-specific manner. In the present study, we sequenced the transcriptome of adult *Onchocerca flexuosa* in order to further explore the phenomenon of horizontal gene transfer between *Wolbachia* and presently *Wolbachia*-free filarial species. We estimate that 40% of all *O. flexuosa* protein-coding genes are represented in our dataset, and we were able to detect regions with homology to 97 different *Wolbachia*-like genes. The transcriptome data facilitated a follow-up proteomic analysis in which 1,800 *O. flexuosa* proteins were identified, including two candidate *Wolbachia*-like proteins. Peptide antibodies raised against the two mass-spectroscopy identified and other computationally predicted *Wolbachia*-like proteins were used to further confirm their expression. Immunohistochemistry studies indicated that these proteins were present in many body regions in adult worms. However, *in situ* hybridization studies showed that the *Wolbachia*-like transcripts are expressed in the lateral chords, the tissues where *Wolbachia* are concentrated in species that harbor the *Wolbachia* endosymbiont. Future studies will attempt to demonstrate the functional significance of remnant *Wolbachia* genes and proteins in *Wolbachia*-negative filarial worms.

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PROTEOMIC ANALYSIS OF EXCRETORY-SECRETORY PRODUCTS OF THE FILARIAL NEMATODE *LITOMOSOIDES SIGMODONTIS*

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The excretory-secretory (ES) products of a parasitic worm represent the 'frontline' in its interaction with the host. These products are known to have immunomodulatory roles in parasite invasion and long-term persistence of infection. The filarial nematode *Litomosoides sigmodontis* is a tractable experimental model for filariasis, as it can produce transmissible offspring in BALB/c mice. Proteomic analysis of adult female *L. sigmodontis* ES products was performed using shotgun LC-MS/MS, identifying several hundred proteins against a draft *L. sigmodontis* genome assembly. The protein abundance profile of the ES differed greatly to that of the somatic protein extract. The predominant ES protein families were protease inhibitors, proteases, lipid-binding proteins and antioxidants. The cysteine protease inhibitor Ls-cystatin, a key vaccine candidate, was the most abundant species present in the ES. Members of the transthyretin-like protein family were also well represented, consistent with earlier studies on the ES of *Ostertagia ostertagi* and *Brugia malayi*. Several previously characterised filarial antigens, including FAR1, leucyl aminopeptidase and RAL2 were also highly enriched in the ES material. In addition, a novel protein product highly expressed in the ES exhibited homology to an apolipophorin from *Ascaris suum* (a lipid-binding protein). However, only three proteins from the *Wolbachia* endosymbiont of *L. sigmodontis* were detected and at low abundance. These initial proteomic data from the adult females will be compared to the ES protein profiles of the adult male, microfilaria and L3 life stages of *L. sigmodontis* to obtain a comprehensive representation of the quantitative changes in the secretome during filarial development.

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IDENTIFICATION OF GENES CONTAINING ECDYSONE RESPONSE ELEMENTS IN THE GENOME OF *BRUGIA MALAYI*

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Recent studies have demonstrated that filarial parasites contain a functional homologue of the insect ecdysone receptor (EcR). As a first step in deciphering the physiological role that ecdysteroids play in filarial parasites, adult female parasites cultured in the presence and absence of 20-OH ecdysone were metabolically labeled. Gel electrophoretic analysis of proteins extracted from the cultured parasites revealed changes in the level of expression of several proteins, indicating that adult female parasites contained an ecdysone-responsive gene network. A bioinformatic analysis was then conducted to identify putative ecdysone response elements (EcREs) in the *B. malayi* genome. A total of 18 genes were identified that contained putative EcREs located in the 4 kbp upstream from the start of their open reading frames. The most common functional classifications of the encoded proteins were factors involved in transcription and metabolism. These genes revealed a number of different developmental patterns of transcription. The promoter of one EcRE-containing gene was cloned into an luciferase reporter vector and transfected into *B. malayi* embryos. Reporter gene expression from embryos transfected with this construct was up-regulated by 20-OH ecdysone, a response which was dependent upon the putative EcRE. These results demonstrate the presence of endogenous functional EcREs in the *B. malayi* genome and provide insights into the role that ecdysteroids may play in the developmental processes of *B. malayi*.

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STRUCTURAL ELUCIDATION OF *WUCHERERIA BANCROFTI* GLUTATHIONE-S-TRANSFERASE BY X-RAY CRYSTALLOGRAPHY TO EVALUATE ITS ROLE AS A THERAPEUTIC TARGET FOR HUMAN LYMPHATIC FILARIASIS

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Human lymphatic filariasis is an incapacitating vector borne disease and is the world's second leading cause of long-term disability. To worsen the condition there are no vaccines yet and vector control programs have limitations of insect resistance. The current drugs have limited ability in removing adult worms and do not remedy chronic morbidity and are suitable only for preliminary control measures. Further their broad use would increase the likelihood of accelerated drug resistance. With this distressing scenario there is a growing demand to identify new molecular targets for lymphatic filariasis towards development of drugs and prophylactics. The current study involved in structurally characterizing filarial glutathione-S-transferase (*Wb*-GST) as a drug target for lymphatic filariasis. Accordingly, the recombinant *Wb*-GST was expressed, purified and co-crystallised along with its native substrate glutathione. The structure was solved at a resolution of 2.3Å by X-ray crystallography. The structure resembles ϖ-class GSTs. The superimposed structures of *Wb*-GST and *Hu*-GST (human host) monomers showed an r.m.s. deviation of 1.2Å for all Cα atoms. The G-site residues were highly conserved (differed by 8%), whereas the H-site residues revealed a significant difference (62%) between *Wb*-GST and *Hu*-GST. The H-site of *Wb*-GST showed greater accessibility for electrophilic substrates compared to *Hu*-GST. The electron

density map of *Wb*-GST showed that the catalytic residue Tyr^7 swings off and works as a proton shuttle for catalytic stabilization. The *Wb*-GST structure also revealed the presence of non-catalytic ligand binding sites (ligandin function) in the intersubunit cleft, which can serve as a binding site for hydrophobic ligands. These crucial insights from structural data could be exploited for developing parasite-specific inhibitors.

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A VALIDATION STUDY FOR A MULTIPLEX QPCR ASSAY FOR THE DETECTION OF *WUCHERERIA BANCROFTI* AND *BRUGIA MALAYI*

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Responsible for causing infection in more than 120 million individuals, *Wuchereria bancrofti* and *Brugia malayi* are the primary causative agents of lymphatic filariasis. As a number of Southeast Asian and South Pacific countries exist that are co-endemic for both parasite species, diagnostic tools capable of simultaneous detection of both filarial nematodes are an attractive option for infection monitoring and surveillance efforts. We previously described the development of a multiplex qPCR assay for the detection of both filarids within a single pool containing DNA extracted from larval worms of both species. However, the usefulness of this assay as a time and money-saving tool is dependent upon the assay's ability to accurately and repeatedly detect parasite DNA extracted from human bloodspots and from mosquito vectors. Here we describe a validation study using both vector mosquito DNA extracts and human bloodspot DNA extracts. This study demonstrates the sensitivity of this multiplex qPCR assay at the 1 pg level, which is as sensitive as the established singleplex assays for the detection of *W. bancrofti* and *B. malayi*.

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HISTAMINE RELEASE DURING *LITOMOSIDES SIGMONDONTIS* INFECTION ENHANCES ADULT WORM BURDEN

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Numerous studies have demonstrated that helminth antigens induce release of histamine from basophils and mast cells of infected hosts. To date, however, the role histamine plays in the immune response against helminths has not been well characterized. In this study, we evaluated the role of histamine in mice infected with ***Litomosoides sigmondontis,*** a tissue-invasive filarial infection of rodents that lives for months in immunocompetent Balb/c mice. Extended time-course studies revealed that histamine in plasma peaked at 8 weeks of infection whereas expression of histidine decarboxylase mRNA in circulating blood cells increased throughout the course of infection. Mice vaccinated with irradiated L3 larvae demonstrated substantial increases in circulating histamine levels 30 minutes after challenge infection, but administration of HR1 and HR2 receptor blockers did not attenuate the protective efficacy of vaccination. Interestingly, short time course measurements demonstrated that primary infection of unvaccinated mice with L3s also causes histamine release into the bloodstream 30 minutes following infection indicating a non-specific mechanism of histamine release. To evaluate the role histamine may play during infection, mice were chronically administered HR1, HR2, and a combination of HR1 and HR2 blockers in their drinking water and assessed for adult worm survival after inoculation with 40 L3 larvae. Surprisingly, at 8 weeks post-infection all groups of mice treated with antihistamine antagonists had significantly reduced numbers of adult worms compared to untreated controls. Taken together, these data indicate that histamine, rather than being involved in vaccine-mediated protection, may be induced by filarial parasites for their growth and/or survival ***in vivo.***

1491

EOSINOPHILS AND STAT6 REGULATE *TRICHINELLA SPIRALIS* MUSCLE INFECTION BY CONTROLLING PARASITE GROWTH

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The parasitic nematode ***Trichinella spiralis*** establishes chronic infection in skeletal muscle. The muscle phase of infection is characterized by tissue and blood eosinophilia. Using two models of eosinophil-ablated mice, we have previously shown that larval growth and survival are significantly compromised in the absence of eosinophils and that this correlates with reduced Th2 immunity. We show here that reduced Th2 cell accumulation at infection sites is caused by impaired Th2 cell production in draining lymph nodes. Defective Th2 cell accumulation did not correlate with the expression pattern of chemokines that direct the migration/activation of T cells, nor the ability of T cells to enter antigen-bearing tissue. Moreover, studies using STAT6-/- and IL-13-/- mice revealed that the IL-4/STAT6 axis regulated parasite growth. Impaired parasite growth in eosinophil-deficient mice correlated with increased expression of genes associated with nutrient deprivation (AMPK and INSR), but neither muscle nor larval glycogen content were affected. Our results support a pivotal immunoregulatory role for eosinophils in acquired immunity and nutrient acquisition during nematode infection.

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EVIDENCE FOR THE SEQUESTRATION OF DEVELOPING *PLASMODIUM FALCIPARUM* GAMETOCYTES IN THE BONE MARROW

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A hallmark of ***Plasmodium falciparum*** infection is the sequestration of asexual stages in deep tissue, which has been linked to cerebral malaria and other disease outcomes. Like late asexual stages, immature sexual stages are visibly absent from the bloodstream and are hypothesized to sequester. However, unlike asexual stages, their localization and mechanism for sequestration is largely unknown. In the current study, we systematically quantified gametocyte sequestration in autopsy cases from an ongoing study of fatal pediatric malaria in Blantyre, Malawi. An organ survey using immunohistochemistry (IHC) on tissue sections from nine body sites (brain, lung, heart, intestine, liver, kidney, subcutaneous fat, spleen, and bone marrow) suggested enrichment of gametocytes in the bone marrow. Quantitative real time RT-PCR supported this finding and revealed transcriptional signatures specific to young gametocytes, confirming that we are in fact observing gametocytes during development. Following up on these significant findings, we performed electron microscopy and observed the presence of knobless parasites in the bone marrow. We are currently performing detailed IHC studies on bone marrow samples using additional tissue markers, and complementary ***in vitro*** experiments to test alternative models of gametocyte development in the human bone marrow. The identification and characterization of a genuine bone marrow cycle of ***P. falciparum*** gametocytes is of

great relevance, in particular considering the fieldβs renewed focus on understanding the dynamics of malaria transmission and the development of new strategies to interrupt it.

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A NOVEL *PLASMODIUM FALCIPARUM* SR PROTEIN IS AN ALTERNATIVE SPLICING FACTOR THAT IS REQUIRED FOR PARASITE PROLIFERATION IN HUMAN ERYTHROCYTES

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The malaria parasites have a complex life cycle, during which it undergoes significant biological changes to adapt to different hosts and changing environments. ***Plasmodium falciparum***, the deadliest form of human malaria, has adapted to its complex life cycle with relatively small number of genes. Alternative splicing (AS) is an important post-transcriptional mechanisms that enables eukaryotic organisms to expand their protein repertoire out of relatively small number of genes. SR proteins are major regulators of splicing in higher eukaryotes. Nevertheless, the splicing as well as the AS machinery in ***Plasmodium spp***. are still elusive. We show that PfSR1 is a putative SR protein that can mediate RNA splicing ***in vitro***. In addition, we demonstrate that PfSR1 functions as an alternative splicing factor in a mini-gene system similar to the mammalian SRSF1. Expression of PfSR1-***myc*** in ***P. falciparum*** shows distinct patterns of cellular localization during intra erythrocytic development. Furthermore, we determine that the predicted RS domain of PfSR1 is essential for its localization to the nucleus. Finally, we demonstrate that proper regulation of ***pfsr1*** is required for parasite proliferation in human RBCs, and affect the splicing pattern of endogenous genes.

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A CELL INTRINSIC ROLE FOR MUC5AC IN MEDIATING THE TH2-RESPONSE TO HELMINTHES AND ALLERGIC ASTHMA

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MUC5AC is a secreted mucin known to be upregulated in response to IL-13 as part of the Th2-mediated response that occurs during helminth infection, fibrotic disease and allergic asthma. IL-13 signals through the Type II IL-4 receptor by binding the IL-13Rα1 chain to drive complex formation and phosphorylation of STAT6, thus mediating downstream effects such as upregulation Fizz1, Ym1 and Arg1, as well as increased eosinophilia, tissue remodeling and mucus hypersecretion. The purpose of this study was to investigate the role of Muc5ac in mouse models of helminth infection, fibrosis and allergic asthma using wildtype C57BL/6 (WT) and Muc5ac knockout (KO) mice. The three models used included a model of *Nippostrongylus brasiliensis* infection, a pulmonary granuloma model using *Schistosoma mansoni* eggs, and a model of allergic asthma using house dust mite. In all three models, a significant reduction in airway eosinophilia in conjunction with reduced expression of Fizz1, Ym1 and Arg1 was observed in KO compared to WT mice; however, no differences in the expression or production of IL-4, IL-5 and IL-13 were observed. To determine if the KO mice were capable of responding to IL-13, rIL-13 was delivered i.t. to WT and KO mice. No tissue inflammation, airway eosinophilia or increase in IL-13 regulated genes was observed in the KO mice in response to rIL-13. Alveolar macrophages and lung fibroblasts isolated from naïve WT and KO mice were grown in culture and treated with either IL-4 or IL-13. Isolated cells from KO mice had reduced expression of Fizz1 and Ym1 compared to WT in response to IL-13, however did not have reduced expression levels of these genes in response to IL-4. Phosphorylation of STAT6 in response to IL-13 but not IL-4 was ablated in KO macrophages, and phosphorylation of STAT6 in response to IL-4 was also ablated after pretreatment with a blocking antibody against the Type I IL-4 receptor. These data identify Muc5ac as a novel component of the Type II IL-4 receptor and thus a novel target to disrupt IL-4/IL-13-mediated inflammation.

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TLR3-DEPENDENT RECOGNITION OF A PROTOZOAN PARASITE

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Despite striking similarities in invasion, intracellular growth and cellular ultrastructure between ***Toxoplasma gondii*** and its close relative ***Neospora caninum***, these two protozoan parasite species exhibit different host ranges and are associated with distinct disease pathogenesis. The molecular mechanisms underlying these differences have not been well characterized. To address this, we utilized comparative genomics of the host response to these parasites in order to identify host pathways induced during ***Neospora***, but not ***Toxoplasma*** infection. Our results revealed that ***Neospora*** is a potent activator of innate immune signaling and canonical antiviral responses, whereas representative members of the three archetypal strains of ***Toxoplasma*** failed to trigger this host response. Recognition of ***Neospora*** by macrophages occurs via ***Tlr3*** and the adapter protein ***Trif***, and is conserved across multiple species and cell types. RNA isolated from ***Neospora***, but not ***Toxoplasma***, is able to induce potent antiviral responses when targeted to the host endosomal system. Surprisingly, we found that although live ***Toxoplasma*** failed to trigger type I interferon production, heat-killed parasites were potent activators of this response. Direct competition experiments between ***Toxoplasma*** and ***Neospora*** revealed that ***Toxoplasma*** potently suppresses innate immune signaling to prevent type I interferon production and that this is the dominant phenotype, suggesting that ***Toxoplasma*** acquired and retained a suppressive factor after divergence from ***Neospora***.

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TOXOPLASMA SUBVERTS HOST CELL IMMUNE RESPONSE VIA ASSOCIATION WITH HOST MITOCHONDRIA

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As with some bacterial pathogens, the tachyzoite stage of the intracellular parasite ***Toxoplasma gondii*** is often found specifically and extensively associated with host mitochondria at the parasitophorous vacuole membrane (PVM) (Jones and Hirsch, 1972; Sinai et al., 1997). Although previously assumed to be metabolically beneficial for the parasite, the actual consequences of host mitochondrial association (HMA) and the molecules that mediate it have not been determined. We have observed

that HMA is substantially diminished in Type II parasites relative to Types I and III. This has enabled us to use genetic analysis of F1 progeny from a cross between Type II and Type III parasites to map the parasite locus involved. Through a candidate gene approach, we have identified the specific gene involved and dubbed it ***Mitochondrial Association Factor 1 (MAF1)***. Introduction of a Type I allele of ***MAF1*** into Type II parasites is sufficient for conferring a strong HMA phenotype and this is associated with dramatic global changes in the host cell's transcriptional and induced cytokine response to parasitic infection. These results support a growing body of literature that mitochondria are a "hub" of innate immune responses. HMA may represent, therefore, an important adaptation by some strains of ***Toxoplasma*** to subvert host immunity, as well as a new mechanism by which an intracellular pathogen can interact with its host and manipulate this interaction.

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CHEW YOUR FOOD: "PARTIAL INGESTION" PLAYS A ROLE IN HUMAN CELL KILLING BY *ENTAMOEBA HISTOLYTICA*

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Entamoeba histolytica is the causative agent of amoebiasis, a diarrheal disease that is a major source of morbidity and mortality in the developing world. Pathogenesis is associated with profound tissue destruction, manifesting as ulceration of the intestinal epithelium or abscesses in extraintestinal sites. The cytotoxic activity of the parasite is central to tissue destruction, but the mechanism by which human cell death is induced is unknown. We sought to elucidate the mechanism by first employing live cell fluorescence video microscopy to observe host-parasite interactions in real time. Surprisingly, we found that within one minute, the amoebae internalize distinct "pieces" of the targeted human cell. These "pieces" contain target cell membrane and cytoplasm and organelles. "Partial ingestion" of the target cell precedes death, as assessed by irreversible calcium elevation and membrane permeabilization. We employed multiple independent strategies to inhibit amoebic phagocytosis in order to determine if ingestion is required for cell killing. By using Amnis Imagestream analysis to simultaneously quantify ingestion and killing, we find that the inhibition of partial ingestion prevents host cell killing. Live two-photon microscopy of amoebae with ***ex vivo*** mouse colon tissue demonstrates that the amoebae also partially ingest enterocytes, suggesting that partial ingestion is relevant to ***in vivo*** tissue destruction. Notably, we have rarely detected complete internalization of the human cells, and we find that once cells have been killed, they are not further ingested by the parasite. Therefore we speculate that complete ingestion of the killed cell may not be the "goal" and that rather partial ingestion represents an unusual, "offensive" mechanism to elicit cell killing. Thus, through these studies we have uncovered surprising host-parasite interactions and are beginning to get a clearer picture of how this parasite effects such devastating tissue destruction.

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A MITOCHONDRIAL CATION/PROTON ANTIPORTER IS ESSENTIAL IN BOTH LIFE STAGES OF *TRYPANOSOMA BRUCEI BRUCEI* AND THE AKINETOPLASTIC *TRYPANONSOMA BRUCEI EVANSI*

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The leucine zipper EF-hand-containing transmembrane protein (Letm1) is a ubiquitous mitochondrial (mt) protein that serves as a cation/proton antiporter across the inner membrane. It remains controversial whether the cation in question is K^+ or Ca^{2+}, as there are data supporting both scenarios. Furthermore, Letm1 is believed to anchor mitoribosomes to facilitate translation of mt genes in yeast. RNAi-silencing of Letm1 in PS and BS ***Trypanosoma brucei brucei***, indicate this protein is essential in both life stages. Its ablation results in mitochondrial swelling, consistent with a role in cation efflux from the matrix. Furthermore, mitochondrial translation is indeed compromised in PS Letm1 knockdowns. However, treatment with ionophores that can mediate K^+/H^+ exchange ameliorates these RNAi phenotypes. Letm1 is also essential in ***T.b. evansi***, where translation is non-existent, not only supporting Letm1's role in K^+/H^+ exchange but also indicating that the energy expenditure needed to maintain an active mitochondrion in the BS, which does not produce energy as in the PS, is cellular ion homeostasis.

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INTERACTION AND COEVOLUTION BETWEEN POLYMORPHIC IRG PROTEIN FAMILY AND *T.GONDII* VIRULENCE FACTORS

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Immunity-related GTPases (IRGs) are important cell-autonomous resistant factors in mice against ***Toxoplasma gondii*** and ***Chlamydia trachomatis***. We will report on significant polymorphism and copy number variation of IRG genes. In this study, the sequences of IRG genes were assembled from 18 strains of mouse from the Sanger Mouse Genomes Project and NIG. Further IRG genes were amplified and sequenced from genomic DNA samples of wild derived mouse strains and wild mice. The results reveal a gene family with haplotypic polymorphism apparently on the scale of the MHC in mouse populations. The ***IRG*** genes are essential for resistance against ***T. gondii*** and are the targets of virulence factors. Our experiments show an interaction between some IRG members and two polymorphic ***T. gondii*** virulence factors, the secreted kinase ROP18 and the secreted pseudokinase ROP5. Together these proteins inactivate IRG proteins by targeted phosphorylation of the switch I loop of the nucleotide binding domain. Certain ***IRG*** haplotypes may confer differential resistance of wild derived mouse strains against virulent ***T. gondii*** strains.

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