## 1

### 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.

## 2

### 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.

## 7

### 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.

## 10

### 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.

## 11

### 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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As part of a mass screening and treatment campaign to reduce malaria transmission in southern Zambia, all individuals residing in the catchment

areas of 10 health facilities were tested for Plasmodium falciparum infections with ICT Mal Pf rapid diagnostic tests (RDT). Implementing reactive case detection to decrease the infectious reservoir of asymptomatic malaria parasite positive individuals requires a better understanding of the spatial distribution of malaria infectivity to maximize testing of positives relative to the total number of community members tested. We used the difference in K functions between RDT+ and RDT- individuals to analyze the spatial distribution RDT+ individuals in all villages and then in villages stratified by parasite prevalence at <5%, 5-39% and >39%. We also assessed the sensitivity and specificity of reactive case detection to find RDT+ individuals without a history of fever by screening all individuals within varying distances of RDT+ individuals with a history of fever among all villages and within the stratified villages. Overall malaria parasite prevalence was found to be 27.3% (95% CI = 26.9% - 27.7%); the distribution of malaria parasite prevalence was extremely heterogeneous ranging from 1.2% - 39.9% within health facility catchment areas. Among all villages RDT+ individuals clustered within households to 25 meters, and then at distances > 175 meters. When stratifying by village parasite prevalence RDT+ individuals clustered within households at all levels of stratification. In <5% village parasite prevalence was clustered from within households and all distances increasing from the household. In villages with parasite prevalence >39% RDT+ individuals were not clustered beyond the household. Sensitivity and specificity of reactive case detection increased and decreased respectively as the size of the screening radius increased, and was only better than randomly testing individuals in villages with <5% malaria prevalence.

## 13

### 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.

## 15

### 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.

## 16

### 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.

## 17

### 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.

## 18

### 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 ($\mu$ = 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.

## 19

### 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.

## 20

### 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.

## 21

### 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.

## 22

### 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.

## 23

### 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.

## 24

### 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.

## 25

### 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.

## 26

### 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.

## 27

### 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.

## 28

### 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.

## 29

### 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.

## 30

### 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.

## 31

### 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.

## 32

### 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.

## 33

### 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.

## 34

### 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.

## 35

### 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-$\gamma^-$, 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.

## 56

### 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.

## 57

### 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 2$^{nd}$, 3$^{rd}$, 4$^{th}$ 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 2$^{nd}$ and 3$^{rd}$ 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.

## 71

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

**Vanja Kovacic**[1], Johan Esterhuizen[1], Inaki Tirados[2], Clement Mangwiro[2], Steven Torr[2], Michael Lehane[1], Helen Smith[1]

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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.

## 74

### 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 $\alpha$, IL-6 and IL-1$\beta$ were induced at 2 and 4 days while IFN $\gamma$ and IL-17 were induced at 4 day. *T. saginata* antigen stimulated IFN $\gamma$ and IL-17 at 4 days in low concentration respect *T. solium* antigen. While IL-10, TNF $\alpha$, IL-6 and IL-1$\beta$ 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 TNF$\alpha$+ 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 $\alpha$-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 $\alpha$-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.

## 102

### 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.

## 103

### 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.

## 104

### 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.

## 105

### 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?

**David I. Pritchard**

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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

**Abdel Nasser Direny**

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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 $\alpha$-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 $\alpha$-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 $\alpha$-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, $\alpha$-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 ($\varpi$=0.54%) and Gambian ($\varpi$=0.56%) populations than in those from the rest of Africa ($\varpi$=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 ($\kappa$=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.